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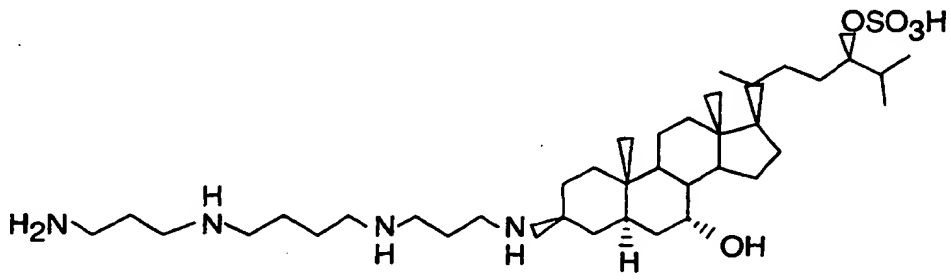

(57) Abstract

A method for controlling (e.g., inhibiting, activating, or up–regulating) various ion channels of cells includes contacting the cell with an effective amount of an aminosterol compound or a salt thereof. This contact can be provided, for example, by administering a pharmaceutical composition to a patient, wherein the pharmaceutical composition includes a pharmaceutically acceptable carrier or excipient, and at least one aminosterol compound. The method according to the invention can be used to treat, for example, ulcers (e.g., gastric ulcers or duodenal ulcers), cardiac arrhythmia, Sjögren's syndrome, cystic fibrosis, head trauma, constipation, post–menopausal dry mucosa syndrome, osteoporosis, or hypertension.

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CERTAIN AMINOSTEROL COMPOUNDS AND USES THEREFOR

RELATED APPLICATION DATA

This application claims priority benefits under 35 U.S.C. § 119 based on U.S. Provisional Patent Appln. No. 60/029,541, filed November 1, 1996, which application is entirely incorporated herein by reference. Additionally, this application relates to U.S. Provisional Patent Appln. No. 60/017,627 and U.S. Patent Appln. No. 08/857,288, each of which is entirely incorporated herein by reference.

BACKGROUND OF THE INVENTION

I. INFORMATION RELATING TO PREVIOUS PATENTS AND APPLICATIONS

Several aminosterol compositions have been isolated from the liver and stomach of the dogfish shark, *Squalus acanthias*. One important aminosterol, squalamine, is the subject of U.S. Patent No. 5,192,756 to Zasloff, et al., which patent is entirely incorporated herein by reference. This patent describes the antibiotic properties of squalamine. Since the discovery of squalamine, several interesting properties of this compound have been discovered. For example, as described in U.S. Patent Appln. Nos. 08/416,883 (filed April 20, 1995) and 08/478,763 (filed June 7, 1995), squalamine may function as an antiangiogenic agent. These patent applications are entirely incorporated herein by reference. Additional uses of squalamine (e.g., as an NHE3 inhibiting agent and as an agent for inhibiting the growth of endothelial cells) are disclosed in U.S. Patent Appln. No. 08/474,799 (filed June 7, 1995). This application also is entirely incorporated herein by reference.

Methods for synthesizing squalamine have been devised, such as the methods described in U.S. Provisional Patent Appln. No. 60/032,378. This application is entirely incorporated

herein by reference. Additionally, U.S. Patent Appln. No. 08/474,799 also discloses squalamine isolation and synthesis techniques.

Stemming from the discovery of squalamine, other aminosterols have been discovered in the dogfish shark liver and stomach and have been investigated. One important aminosterol that has been isolated and identified has the structure shown in Fig. 1a. In this application, the compound having the structure shown in Fig. 1a will be referred to as "compound 1436" or simply "1436." This compound has the general molecular formula $C_{37}H_{72}N_4O_5S$ and a calculated molecular weight of 684.53017. Other aminosterols, including squalamine, are shown in Figs. 1b to 1g.

Compound 1436, as well as other aminosterols, previously has been described in U.S. Patent Appln. Nos. 08/483,057 and 08/487,443, each filed June 7, 1995 and U.S. Patent Appln. No. 08/857,288 filed on May 16, 1997. Each of these U.S. patent applications is entirely incorporated herein by reference. These U.S. patent applications describe the structure of compound 1436 and other aminosterols, as well as processes for synthesizing and isolating compound 1436 and other aminosterols. For example, compound 1436 may be prepared from a squalamine starting material. As further described in these patent applications, compound 1436 has a variety of interesting properties. For example, compound 1436 has been found to be capable of inhibiting human T-lymphocyte proliferation, as well as being capable of inhibiting the proliferation of a wide variety of other cells and tissues.

It also has been found that compound 1436 has antiviral effects on a wide variety of viruses. For example, compound 1436 has been found to inhibit replication of the human immunodeficiency virus ("HIV") in accepted models. In addition to its inhibitory effect on HIV,

compound 1436 also inhibits replication of the simian immunodeficiency virus ("SIV") and the herpes simplex virus ("HSV"). Based on these properties, it has been concluded that compound 1436 has immunomodulatory effects.

In addition to its favorable antiviral activity, it has been found that compound 1436 also has anti-proliferative effects that may assist in the treatment of various types of cancer. The growth of various different types of cancer cells are inhibited by treatment with compound 1436, for example, the proliferation of human melanoma cells as well as murine acute lymphocytic leukemia (murine "ALL") and human myeloid leukemic cells.

It has been found that arthritis also may be effectively treated using compound 1436. In mouse models, the use of compound 1436 was found to significantly reduce paw swelling in mice that were induced to develop arthritis. This property of compound 1436 is described, for example, in U.S. Patent Appln. No. 08/857,288.

In addition to treating various ailments and diseases, compound 1436 also may be used to reduce weight gain in mammals. The weight gain of the animals in these studies was controlled by appetite suppression and reduction in growth hormone production. The animals continued to have normal fluid consumption and were healthy, viable animals. Thus, compound 1436 may be used as a dietary aid to assist one in maintaining weight control in a healthy manner. Based on studies relating to weight gain, applicants have concluded that compound 1436 has metabolic effects and hormonal effects. These studies, as well as the diuretic effects of compound 1436, are described in U.S. Patent Appln. No. 08/857,288 mentioned above.

Applicants also have found that compound 1436 can selectively inhibit certain sodium/proton exchangers (also called "NHEs" or "Na/proton exchangers" in this application).

Several different isoforms of NHE are known to exist in mammals (e.g., NHE1, NHE2, NHE3, NHE4 and NHE5). Compound 1436 has been found to specifically inhibit NHE3 and not NHE1 or NHE2. Accordingly, compound 1436 may be used for treatment of proliferation or activation dependent conditions that rely on the function of NHE3, such as cancer, viral diseases, and ischemic reprofusion injury.

II. INFORMATION RELATING TO THIS APPLICATION

Compound 1436 and other aminosterol compounds isolated from the dogfish shark liver and stomach and their analogues have been found to possess interesting antibiotic and anti-proliferative properties with respect to wide variety of cells and tissues. These interesting properties of compound 1436 and other aminosterols have prompted applicants to conduct further investigations into the uses and properties of compound 1436 and these other aminosterols. This investigation has led to the discovery that compound 1436 activates the calcium 2^+ ("Ca^{2+}") - activated chloride efflux channel of cells. It also was determined that compound 1521 could antagonize that activation. Compounds 1360A and 1361A were found to selectively inhibit various anion exchange (AE) transporters. The modulation of the above channels and transporters can be used to treat certain diseases and conditions enumerated below.

SUMMARY OF THE INVENTION

This invention relates to pharmaceutical compositions, including a compound according to formula 1436 as shown in Fig. 1a or the other aminosterols shown in Figs. 1b-1g, or pharmaceutically acceptable salts thereof (as an active ingredient), and a pharmaceutically acceptable carrier or excipient. The invention further relates to pharmaceutical products

including the pharmaceutical composition described above. Such pharmaceutical products may be provided for the treatment of gastric and duodenal ulcers; cardiac arrhythmia; cystic fibrosis; osteoporosis; constipation; post-menopausal dry mucosa syndrome; head trauma; Sjögren's syndrome; or hypertension. Gastric and duodenal ulcers could be treated by the inhibition of the Cl^- channel which would reduce acid secretion in the stomach and duodenum. Cystic fibrosis could be alleviated by activation of a Cl^- channel to compensate for defective Cl^- channels associated with the disease. Osteoporosis can be treated by activation of Ca^{++} efflux in bone marrow cells. Activation of Cl^- efflux also produces water secretion, which can be useful for the treatment of Sjögren's syndrome, constipation, and post-menopausal dry mucosa syndrome. The inhibition of water secretion can be used to treat edema in head trauma. Blockers of Ca^{++} channels, such as compound 1521, could be used to treat cardiac arrhythmia and hypertension.

This invention further relates to various methods for using the pharmaceutical compositions in accordance with the invention. In the methods according to the invention, various diseases or symptoms of diseases or ailments are treated by administering an effective amount of the above-described pharmaceutical compositions. "Treat," "treated," or "treating," as used in this application, may mean complete elimination of the disease, ailment, or symptoms, or it may mean reducing, suppressing, or ameliorating the severity of the disease, ailment, or symptoms. As examples, cardiac arrhythmia, hypertension, gastric ulcers, duodenal ulcers, Sjögren's syndrome, constipation, post-menopausal dry mucosa syndrome, head trauma, and cystic fibrosis may be treated by administering an effective amount of the pharmaceutical compositions in accordance with the invention. Additionally, certain body functions may be controlled (e.g., up regulated or inhibited) by administering an effective amount of the above-

described pharmaceutical compositions. For example, in this manner, the Ca^{2+}-activated chloride efflux channel can be activated or inhibited, or AE-mediated anion exchange can be inhibited, by administering an effective amount of the pharmaceutical compositions in accordance with the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantageous aspects of the invention will be evident from the following detailed description which should be considered in conjunction with the attached drawings, wherein:

Figs. 1a to 1g illustrate the molecular structure of various aminosterol compounds;

Figs. 2a and 2b illustrate the effect of 1436 on Cl^- efflux and influx;

Fig. 3 is a graph that illustrates that 1436 activates the chloride efflux channel;

Figs. 4a and 4b illustrate that 1436 is inactive when injected into oocytes;

Figs. 5a and 5b show the effect of calcium ions on the activity of compound 1436;

Figs. 6a and 6b illustrate the effects of injected EGTA and BAPTA on the activity of compound 1436 ("EGTA" stands for ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid, and "BAPTA" stands for 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid);

Fig. 7 is a graph showing that 1436 activates the ^{45}Ca-efflux channel;

Figs. 8a through 11b show the effect of compound 1436 on the Ca-efflux channel and the chloride efflux channel under a variety of conditions;

Fig. 12 shows the dose response of 1436 in the absence of calcium ions;

Fig. 13 is a chart that shows the effect of various aminosterols on chloride efflux;

Fig. 14 is a graph that illustrates the effect of compound 1521 on the activity of compound 1436 with respect to Cl^- efflux;

Fig. 15 illustrates that in the presence of compound 1521, DIDS activates the chloride efflux channel;

Fig. 16 demonstrates the effect of guanine nucleotides on the activity of compound 1436;

Fig. 17 shows AE1 and AE2 inhibition by various aminosterol compounds;

Fig. 18 is a graph that illustrates inhibition of AE-mediated Cl^- influx by compound 1361;

Fig. 19 is a graph illustrating AE2 inhibition as a function of compound 1360 dosage;

Fig. 20 shows ID_{50} values of aminosterols for $^{36}Cl^-/Cl^-$ exchange;

Figs. 21 through 28 show a schematic diagram for a voltage clamp device and various graphs illustrating the effect of compound 1436 on cells under various conditions;

Figs. 29a and 29b demonstrate the change in oocyte pH after acid loading in the absence of compound 1436 and in the presence of compound 1436; and

Fig. 30 shows the effect of compound 1436 on pH recovery in *Xenopus* oocytes.

Fig. 31 shows a pharmacokinetic study of 1436 in blood plasma after s.q. administration.

DETAILED DESCRIPTION OF THE INVENTION

As described above, compound 1436 and other aminosterol compounds have been discovered in and isolated from the liver and stomach of the dogfish shark. In addition, analogues of these aminosterols have been synthesized as described above. Some aminosterols were noted to induce changes in cell shape and (apparent) size. In particular, compound 1361 was found to alter red blood cell shape. This finding led to testing of aminosterols as inhibitors

of AE (band 3 - related) anion exchange channels. In the process of testing these aminosterols against AE1 and AE2, it was discovered that compound 1436 could activate the Ca^{++}-activated chloride efflux channel.

The invention will be described below in terms of various specific examples and preferred embodiments. These examples and embodiments should be considered to be illustrative of the invention, and not as limiting the same.

EXAMPLE 1

Compound 1436 Effects on Ca^{2+}-Activated Chloride Efflux Channel

In addition to the various NHE pumps which have been shown previously to be affected by squalamine and other aminosterols, cells contain other pumps, carriers, channels, and the like that cells use to regulate various characteristics and functions of the cells. Tests were performed on compound 1436 to determine its effect on the Ca^{2+}-activated chloride efflux channel. It was found that 1436 activates this efflux channel. The test results will be described below.

Notably, compound 1436 could not be evaluated as an anion exchange (AE) channel inhibitor because it activated an endogenous $^{36}Cl^-$ efflux response in both water-injected and native *Xenopus* oocytes.

Figs. 2a and 2b demonstrate the effect of compound 1436 on $^{36}Cl^-$ efflux and influx in *Xenopus* oocytes. In each figure, the concentration of the added 1436 was 2 μM. Compound 1436 was found to activate the chloride efflux channel. This activation occurred without trans-anion requirement, as illustrated in Fig. 3.

When injected into *Xenopus* oocytes, however, compound 1436 is inactive. Note Figs. 4a and 4b.

Figures 5a and 5b demonstrate that calcium ions ("$[Ca^{++}]_i$") improve the action of compound 1436. The 1436 concentration is 2 μM in each instance. The injection of EGTA or BAPTA into the oocyte, which ties up free intracellular Ca^{++} ions, has been found to inhibit the action of compound 1436 on chloride efflux. See also Figs. 6a and 6b.

Applicants have found that compound 1436 activates endogenous Ca^{++} signaling in *Xenopus* oocytes. As shown in Fig. 7, compound 1436 activated the $^{45}Ca^{++}$ efflux channel when administered in a concentration of 2 μM. Additional graphs and charts are provided in Figs. 8a to 12 to show the effect of 1436 on the $[Ca^{++}]_i$ and Cl^- efflux pathways of oocytes under various conditions.

The table of Fig. 13 describes the effects of the various compounds illustrated in Figs. 1a to 1g on oocyte Cl^- efflux in ND-96 and nominally Ca^{++} free ND-96. ND-96 is a balanced salt buffer solution for oocytes that contains (in mM): 96 NaCl, 2KCl, 1.8 $MgCl_2$, 1 $CaCl_2$, and 5 HEPES (Na), pH 7.40. Notably, compound 1436 is active under both of these conditions.

Compound 1521 acts as an antagonist of the 1436-activated Cl^- efflux. This effect is illustrated in Fig. 14. Notably, compound 1436 produces a high Cl^- efflux when present alone at a concentration of 150 nM. When this concentration of compound 1436 is present together with 2μM compound 1521, however, the Cl^- efflux is dramatically reduced, approximately to the efflux level observed when compound 1521 is present alone. In the prior presence of compound 1521, surprisingly, the subsequent addition of DIDS, which is a known inhibitor of Cl^- channels, activates the Cl^- efflux, even in the presence of compound 1436. See Fig. 15.

Fig. 16 illustrates the effects of guanine nucleotides on compound 1436 action. Applicants conclude from this figure that GTP-γ-S may inhibit 1436-mediated activation of Cl^- efflux, but GDP-β-S may be inactive.

To summarize the results of the tests with oocytes, the following conclusions can be made: (1) 1436 activates Cl^- efflux without a trans-anion requirement; (2) 1436 activated Cl^- efflux requires intracellular free Ca; (3) lag time for 1436 activated Cl^- efflux is greatly reduced by removal of Ca_o; (4) injected 1436 is inactive; (5) 1436 slowly elevates intracellular [Ca] in parallel with elevated Cl^- efflux; (6) removal of Ca_o greatly reduces lag time in 1436-mediated elevation of $[Ca]_i$, while (preliminarily) lowering peak $[Ca]_i$; (7) 1436 activates $^{45}Ca^{++}$ efflux; (8) 1436 activated Cl^- efflux is inhibited by DIDS (a known inhibitor of the Cl^- efflux channel), and inhibition is attenuated by removal of Ca_o (DIDS does not block 1436-activated $^{45}Ca^{++}$ efflux); (9) in the absence of Ca_o, heparin injection blocks 1436-induced elevation in the intracellular Ca^{++} level, but it does not block either 1436 activated Cl^- efflux or $^{45}Ca^{++}$ efflux (heparin sulfate has no effect on any of these parameters); and (10) neither extracellular nor intracellular 1436 at 2 μM produces oocyte maturation over two days or longer (apparently non-toxic). From these findings, applicants believe that compound 1436 either (a) interacts with a surface receptor that elevates intracellular Ca^{++} as part of its signaling mechanism, or (b) interacts directly with an exofacial domain of the Ca^{++} regulated Cl^- channel or an associated protein.

EXAMPLE 2

Additional Activity of Aminosterol Compounds on Cells

The aminosterols in Figs. 1a to 1g have differing effects as antagonists of the heterologous AE-mediated anion exchange ($^{36}Cl^-$ efflux) in *Xenopus* oocytes. The test results are

illustrated in Fig. 17. For the $^{36}Cl^-$ efflux experiments described in Fig. 17, defolliculated oocytes were injected with cRNA encoding AE1 or AE2. Forty-eight or more hours later, the oocytes were acutely injected with $Na^{36}Cl$, and efflux of the isotope was monitored into ND-96 in the absence or presence of the indicated aminosterols. Each $^{36}Cl^-$ efflux experiment represents the indicated number of oocytes monitored, first in the absence of the aminosterol compound, and then after exposure to a 2 μM concentration of the noted aminosterol from Figs. 1a to 1g (compound 1508, as shown in Fig. 1e, is a putative polyamine oxidase metabolite of 1436, and compound 1521 (Fig. 1f) can be characterized as a desulfated version of compound 1436). After exposure to the aminosterol, the system was allowed to achieve a steady-state efflux rate.

Squalamine did not affect the mouse AE1- or AE2-mediated $^{36}Cl^-$ fluxes of the *Xenopus* oocytes. Note Fig. 17. Compound 1437 (hydroxymethylsqualamine), however, substantially inhibited AE2 activity (by about 43%), and notably, it had little effect on the AE1 activity.

Compounds 1360 and 1361, on the other hand, each dramatically inhibited AE2-mediated efflux (by about 74% and about 83%, respectively), and these compounds had relatively little effect on AE1 efflux (about 15% and 28%, respectively). Thus, compounds 1360 and 1361 inhibit anion exchange such that they exhibit greater inhibitory activity against AE2 as compared to AE1.

In view of these observations, further studies with compounds 1360 and 1361 were completed. For the test results illustrated in Fig. 18, oocytes were injected with water or with cRNAs encoding mouse AE1 or AE2. Forty-eight hours after injection, the oocytes were preincubated for 60 minutes in ND-96. The ND-96 included 2 μM 1361 aminosterol in some tests and no 1361 aminosterol in other tests.

During the subsequent 15 minutes after the preincubation period, the $^{36}Cl^-$ influx was measured under the same extracellular conditions. Under these prolonged exposure conditions, 1361 inhibited AE1 by 46% and AE2 by 53% (in the graph of Fig. 18, the " * " indicates that $p<0.05$). This essentially equivalent inhibition of AE1 and AE2 at long exposure times differs from the 8-10 fold greater potency displayed for AE2 inhibition when measured at short exposure times, as shown in Fig. 17.

Accordingly, from the tests shown in Figs. 17 and 18, applicants conclude that compound 1361 inhibits AE2 more potently than AE1. This compound ranks with or exceeds in potency most known AE2 inhibitors. Prolonged exposure to 1361 tends to equalize its inhibitory effect on AE1 and AE2.

Fig. 19 is a graph that illustrates the 1360 dose response for inhibiting the AE2-mediated $^{36}Cl^-$ efflux channel in oocytes. Oocytes were injected with cRNA encoding mouse AE2. Forty-eight hours later, the oocytes were acutely injected with $Na^{36}Cl$. ^{36}Cl efflux was monitored, first in the absence of compound 1360, and then in the presence of the indicated increasing concentrations of compound 1360. Efflux rate constants were calculated by linear regression of the efflux curves, and percent inhibition was calculated as follows:

%Inh. = 100 × [1 - (rate constant with 1360 present / rate constant with 1360 absent)].

Notably, even at relatively low 1360 dosages (about 500 nM), about 50% or more Cl^- efflux inhibition was observed. Applicants conclude from this data that 1360 inhibits AE2 in a manner consistent with interaction at a single site.

ID_{50} values for compounds 1360 and 1361 were measured for $^{36}Cl^-/Cl^-$ exchange in *Xenopus* oocytes. Values for both AE1 and AE2 anion exchange were measured. See Fig. 20.

Each $^{36}Cl^-$ efflux experiment noted in Fig. 20 represents one oocyte monitored, first in the absence of any aminosterol, and then after exposure to the noted aminosterol compound in at least three graded concentrations. Each successive concentration was maintained until achievement of a new, steady-state efflux rate. This steady-state condition always was reached within about 16 minutes. All of the experiments described in conjunction with Fig. 20 included a final exposure to DIDS, at a concentration of 200 μM, to confirm the integrity of the carrier-mediated Cl^- transport. These ID_{50} values were derived from a non-linear fit to a hyperbolic inhibition curve.

EXAMPLE 3

Effect of 1436 on Membrane Conductance and Current

A schematic diagram of a voltage-clamp recording apparatus is illustrated in Fig. 21. Current, conductance and potential measurements were made on cell systems in the presence of compound 1436, under various experimental conditions, as shown in Figs. 22-28.

Oocyte pH_i regulates AE2 activity, and oocyte NHE regulates pH_i. Accordingly, applicants tested the effect of compound 1436 on the Na^+/H^+ exchange activity of the oocyte. Figs. 29a and 29b illustrate pH_i traces of oocyte pairs that are allowed to recover from NH_4Cl-induced acid loading in the absence (Fig. 29a) and in the presence (Fig. 29b) of the aminosterol 1436. First, the oocytes were preloaded with a pH sensitive radiometric dye acetoxymethyl ester, BCECF-AM. Loaded oocytes were acidified by incubation in 20mM NH_4Cl, and then allowed to recover. As shown in Fig. 29a, when compound 1436 is not present, pH recovery is relatively rapid. In the presence of 2 μM compound 1436, as shown in Fig. 29b, the pH remains low.

Additional pH_i data is included in Fig. 30. From this data, applicants conclude that compound 1436 inhibits oocyte Na/H exchange that is activated by acid loading.

Squalamine has been found to be an inhibitor of mammalian NHE3-mediated Na/H exchange. As shown above, applicants have examined the activity of aminosterols on cation exchange and anion exchange in Xenopus oocytes. The endogenous Na/H exchanger of Xenopus oocytes mediates ~60% of recovery from an ammonium chloride-induced acid load, as defined by blockade of pH_i recovery by extracellular Na^+ removal or by 100 μM amiloride. Compound 1436 (2 μM) inhibited oocyte pH_i recovery from acid load by 88% (dpH_i/dt = 0.034 ± 0.002 min-1 for control and 0.004 ± 0.003 min-1 for 1436), without apparent lag in onset of action.

From the information set forth in this application, including Figs. 2-30, applicants have reached several conclusions:

(a) Compound 1361 inhibits AE2 more potently than AE1;

(b) Xenopus oocytes express surface membrane receptors for some aminosterol compounds, and it appears that compound 1436 is the ligand of highest apparent potency;

(c) The binding of compound 1436 to its receptor leads to activation of a DIDS-sensitive Cl^- channel and to DIDS-sensitive Cl^- efflux;

(d) The binding of compound 1436 to its receptor also leads to elevation of intracellular Ca^{++} and to increased Ca^{++} efflux;

(e) The reduction of extracellular Ca^{++} accelerates both elevation of intracellular Ca^{++} and Cl^- efflux activated by 1436;

(f) Intracellular heparin inhibits 1436-mediated elevation of intracellular Ca^{++}, but it does not inhibit 1436-mediated Cl^- efflux;

(g) Complete removal of extracellular Ca^{++} abolishes elevation of intracellular Ca^{++} by 1436, but it has no further effect on 1436-activated Cl^- efflux;

(h) GTP-γ-S may inhibit 1436-mediated activation of Cl^- efflux, but GDP-β-S may be inactive;

(I) Reduction of extracellular Ca^{++} and substitution of I^- for Cl^- both decrease inhibition by DIDS of 1436-activated Cl^- efflux;

(j) Most, but not all tested aminosterols show Cl^- efflux agonist activity in low extracellular Ca^{++};

(k) Compound 1521 is an antagonist of 1436-activated Cl^- efflux;

(l) In the prior presence of compound 1521, subsequent addition of DIDS activates Cl^- efflux, even in the presence of compound 1436;

(m) Compound 1436 potently and nearly immediately inhibits the endogenous Na^+/H^+ exchanger of the Xenopus oocyte. This inhibition is much more rapid than that previously observed for mammalian NHE3. Inhibition is observed when Na/H exchange activity is monitored by dpH_i/dt or by ^{22}Na influx, and whether exchange is activated by acid load or by hypertonic shrinkage; and

(n) These and other actions of the aminosterols in Xenopus oocytes and other tissues suggest that at least part of their action derives from interaction with a novel class of plasmalemmal aminosterol receptors.

Blockers of the nonerythroid AE channels, such as the aminosterols 1360 and 1361 have many potential uses. For example, they may be used in the treatment of gastric and duodenal ulcers by inhibition of gastric lumenal acid secretion. Activation of the calcium activated chloride channel, as demonstrated by 1436, may be useful, for example, in the treatment of Sjögren's syndrome or cardiac arrhythmia.

III. THERAPEUTIC ADMINISTRATION AND COMPOSITIONS

The mode of administration of compound 1436 and the other aminosterol compounds may be selected to suit the particular therapeutic use. Modes of administration generally include, but are not limited to, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, inhalation, intralesional, endothelial and oral routes. The compounds may be administered by any convenient route, for example, by infusion or bolus injection, or by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.), and the active aminosterol ingredient may be administered together with other biologically active agents. Administration may be local or systemic.

In this application, the abbreviation "s.q." or "s.c." is used to represent subcutaneous administration of compound 1436 or other substances. The abbreviation "i.p." is used to represent intraperitoneal administration of compound 1436 or other substances. The abbreviation "i.v." is used to represent intravenous administration of compound 1436 or other substances. The abbreviation "i.m." is used to represent intramuscular administration of compound 1436 or other substances.

The present invention also provides pharmaceutical compositions that include compound 1436 or another aminosterol compound as an active ingredient. Such pharmaceutical

compositions include a therapeutically effective amount of compound 1436 (or a pharmaceutically acceptable salt thereof) or another aminosterol compound (or a pharmaceutically acceptable salt thereof) and a pharmaceutically acceptable carrier or excipient. Examples of such a carrier include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The particular form and formulation of the pharmaceutical composition should be selected to suit the mode of administration.

The pharmaceutical composition, if desired, also may contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The pharmaceutical composition may be in any suitable form, such as a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The pharmaceutical composition also may be formulated as a suppository, with traditional binders and carriers, such as triglycerides. Oral formulations may include standard carriers, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Various delivery systems are known and may be used to administer a therapeutic compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules and the like.

In one embodiment, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to humans. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical composition also may include a solubilizing agent and a local anesthetic to ameliorate pain at the cite of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example,

as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the pharmaceutical composition is to be administered by infusion, it may be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

The amount of the therapeutic compound of the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and this amount can be determined by standard clinical techniques known to those skilled in the art through routine experimentation. The precise dose to be employed in the pharmaceutical composition also will depend on the route of administration and the seriousness of the disease or disorder, and should be decided according to the judgement of the practitioner and each patient's circumstances. Effective therapeutical doses may be determined from extrapolations of dose-response curves derived from *in vitro* or animal-model test systems.

The following dosage ranges are exemplary. Suitable dosages for intravenous administration are generally about 20 micrograms to 40 milligrams of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 mg/kg body weight to 1 mg/kg body weight. Suitable dosage ranges for topical administration are generally at least about 0.01% by weight. Suitable dosages for oral administration are generally about 500 micrograms to 800 milligrams per kilogram body weight, and preferably about 1-200 mg/kg body weight. Suppositories generally contain, as the active

ingredient, 0.5 to 10% by weight of the aminosterol active ingredient. Oral formulations preferably contain 10% to 95% active ingredient.

Exemplary dosages of the aminosterol active ingredient for most pharmacological or therapeutical uses fall within the range of about 0.01 mg/kg body weight to about 100 mg/kg body weight. Preferred dosages are from 0.1 to 25 mg/kg body weight.

For subcutaneous administration, applicants have performed a pharmacokinetics study of the administration of compound 1436 in a mouse model. For this test, compound 1436 was administered s.q. at a dose of 10 mg/kg in mice. Fig. 31 shows the blood plasma levels of compound 1436 at various times after this s.q. administration. As shown in Fig. 31, the peak 1436 concentration in the blood plasma from this 10 mg/kg dose was about 175 μg/ml after a time of about 2 hours. After 48 hours, the 1436 concentration is still about 10-15 μg/ml. This data indicates that relatively small 1436 doses may be used for s.q. administration. This data also provides an indication that oral dosing of 1436 will be effective.

The invention also may include a pharmaceutical pack or kit including one or more containers filled with pharmaceutical compositions in accordance with the invention. Associated with such containers may be a notice in the form prescribed by a government agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

By the term "effective amount" in this application, applicants refer to a suitable amount of the active ingredient of the invention, with an appropriate carrier or excipient, including a sufficient amount of the active ingredient to provide the desired effects or results. The effective amount can be readily ascertained by those skilled in the art through routine experimentation.

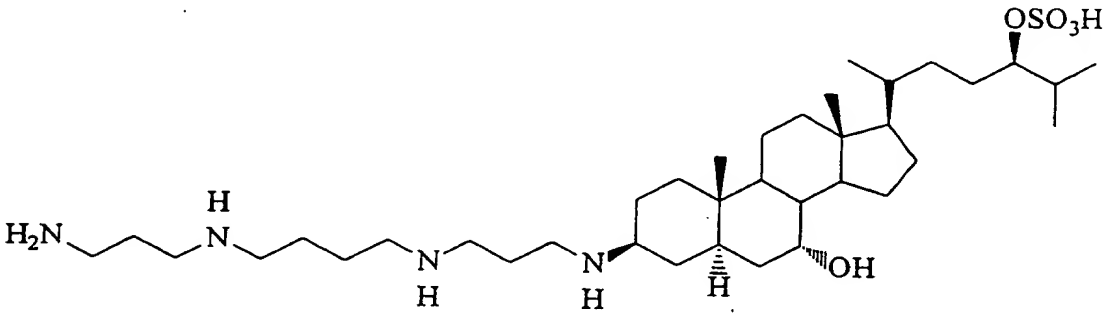
In describing the invention, applicant has stated certain theories in an effort to disclose how and why the invention works in the manner in which it works. These theories are set forth for informational purposes only. Applicants do not wish to be bound by any specific theory of operation.

While the invention has been described in terms of various specific preferred embodiments and specific examples, those skilled in the art will recognize that various changes and modifications can be made without departing from the spirit and scope of the invention, as defined in the appended claims.

WE CLAIM:

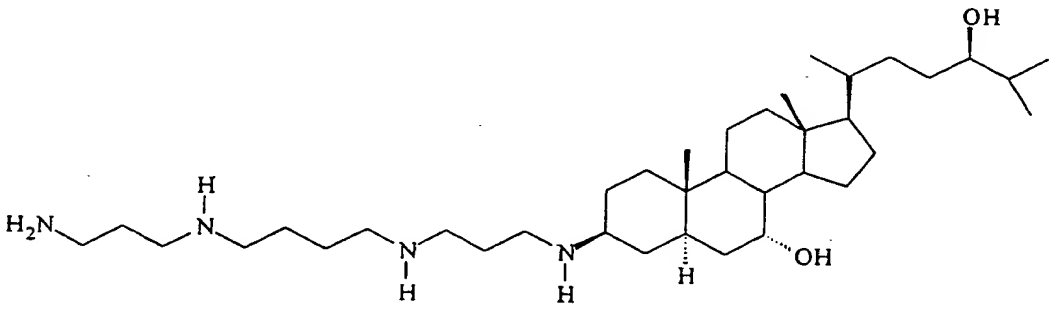
1. A process for controlling a Cl^- efflux channel of a cell, comprising:

contacting the cell with a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound according to formula 1436:



or a pharmaceutically acceptable salt thereof.

2. A process according to claim 1, further comprising contacting the cell with a second compound, wherein the second compound corresponds to formula 1521:

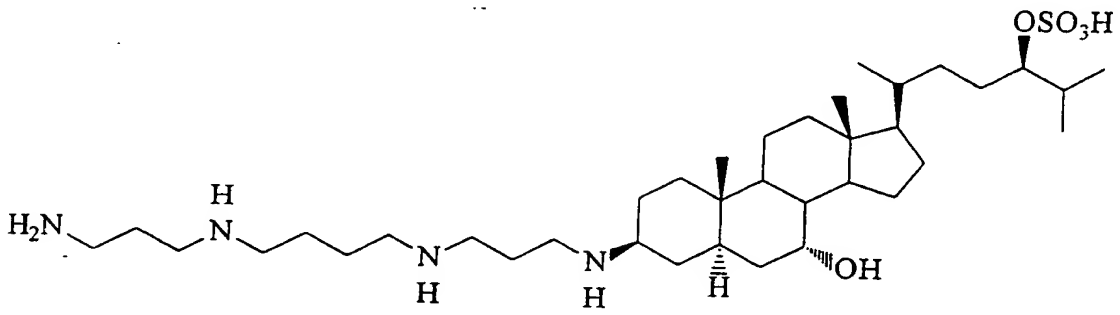


or a pharmaceutically acceptable salt thereof.

3. A process according to claim 1, wherein the contacting step takes place in the presence of calcium or calcium ions.

4. A process for controlling a calcium efflux channel of a cell, comprising:

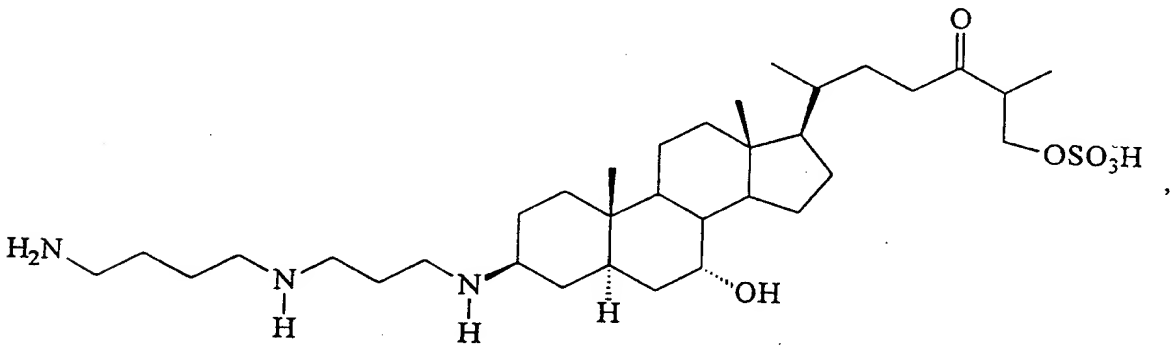
contacting the cell with a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound according to formula 1436:



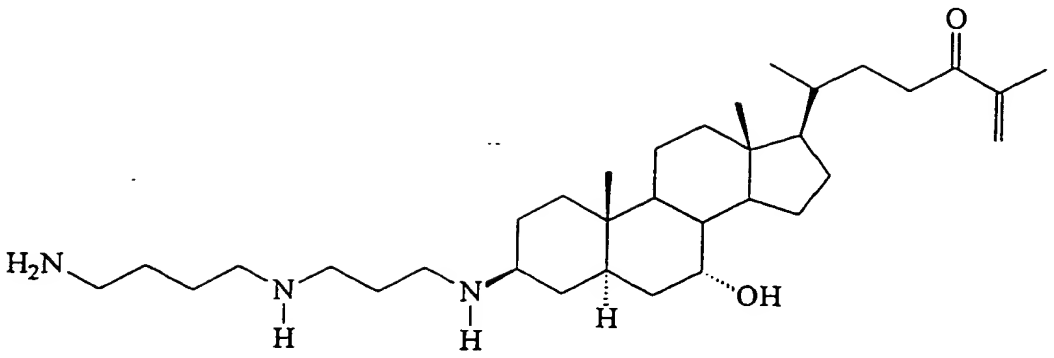
or a pharmaceutically acceptable salt thereof.

5. A process for controlling an anion exchange transporter of a cell, comprising:

contacting the cell with a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound selected from the group consisting of a compound according to formula 1360A as follows:



a pharmaceutically acceptable salt of compound 1360A, a compound according to formula 1361A as follows:



, and

a pharmaceutically acceptable salt of compound 1361A.

6. A process according to claim 5, wherein the compound is compound 1360A or a pharmaceutically acceptable salt thereof.

7. A process according to claim 5, wherein the compound is compound 1361A or a pharmaceutically acceptable salt thereof.

8. A process according to claim 5, wherein the anion exchange transporter is AE1.

9. A process according to claim 5, wherein the anion exchange transporter is AE2.

10. A process according to claim 6, wherein the anion exchange transporter is AE1.

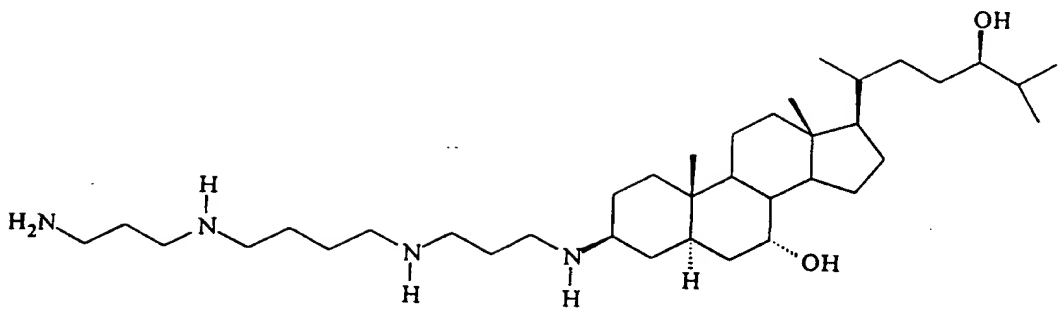
11. A process according to claim 6, wherein the anion exchange transporter is AE2.

12. A process according to claim 7, wherein the anion exchange transporter is AE1.

13. A process according to claim 7, wherein the anion exchange transporter is AE2.

14. A process for controlling a Cl^- efflux channel of a cell, comprising:

contacting the cell with a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound according to formula 1521:

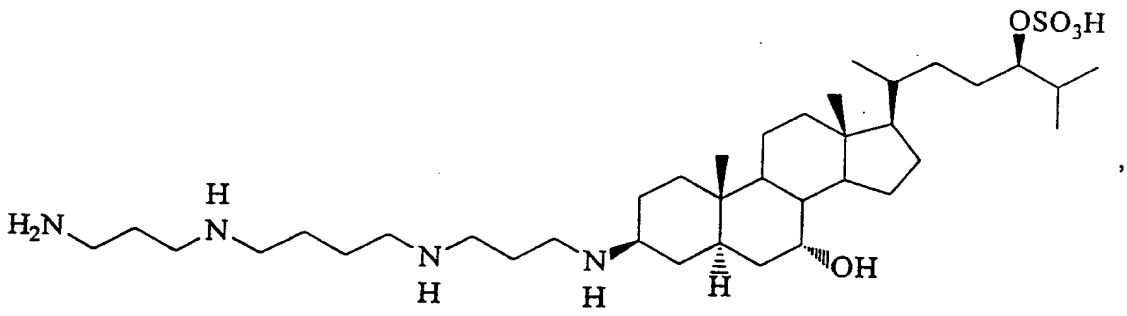


or a pharmaceutically acceptable salt thereof.

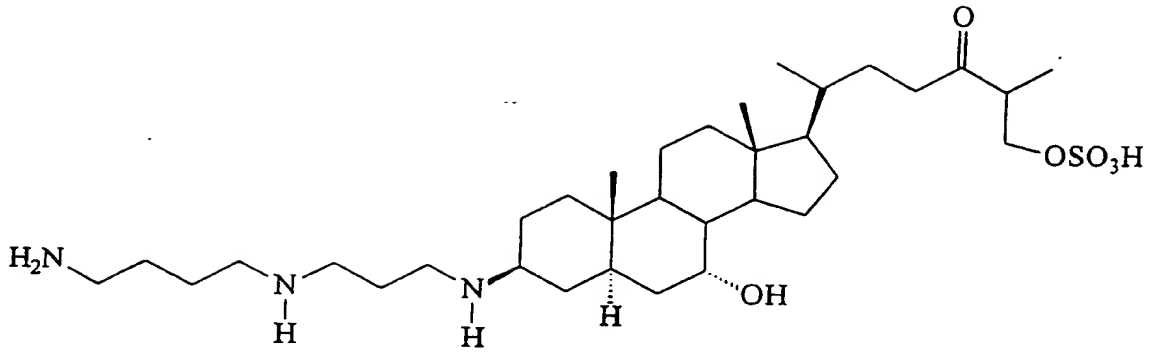
15. A process according to claim 14, further comprising contacting the cell with compound 1521 in the presence of DIDS.

16. A method for treating an ulcer in a patient, comprising:

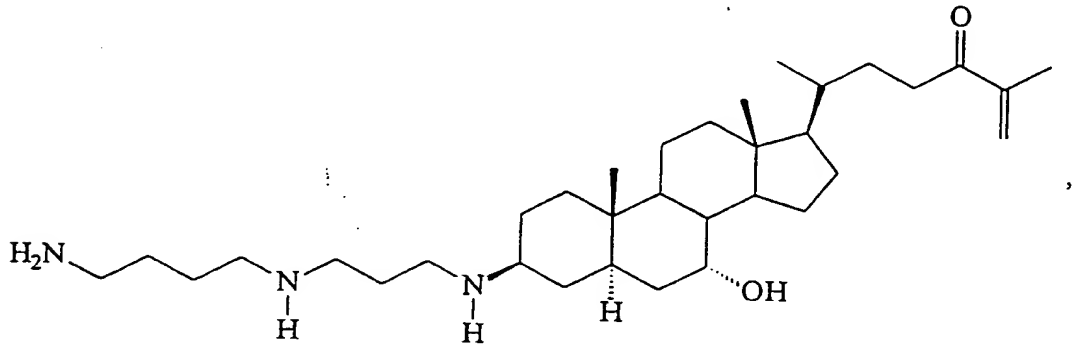
administering to the patient an effective amount of a pharmaceutical composition, wherein the pharmaceutical composition includes a pharmaceutically acceptable carrier or excipient, and a compound selected from the group consisting of: a compound according to formula 1436 as follows:



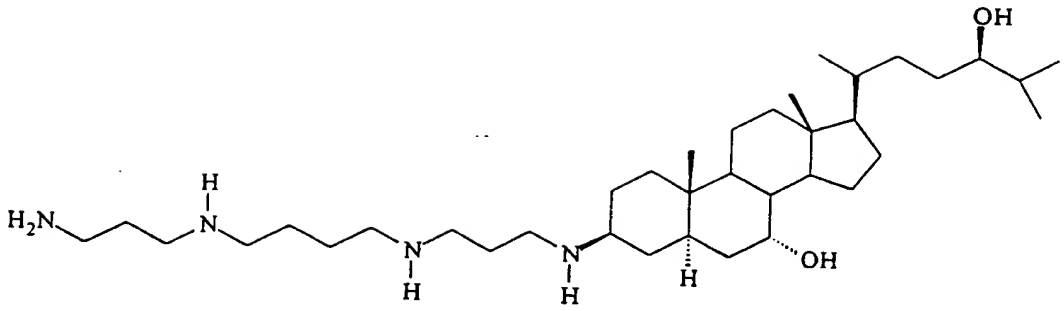
a pharmaceutically acceptable salt of compound 1436, a compound according to formula 1360A as follows:



a pharmaceutically acceptable salt of compound 1360A, a compound according to formula 1361A as follows:



a pharmaceutically acceptable salt of compound 1361A, a compound according to formula 1521 as follows:



, and

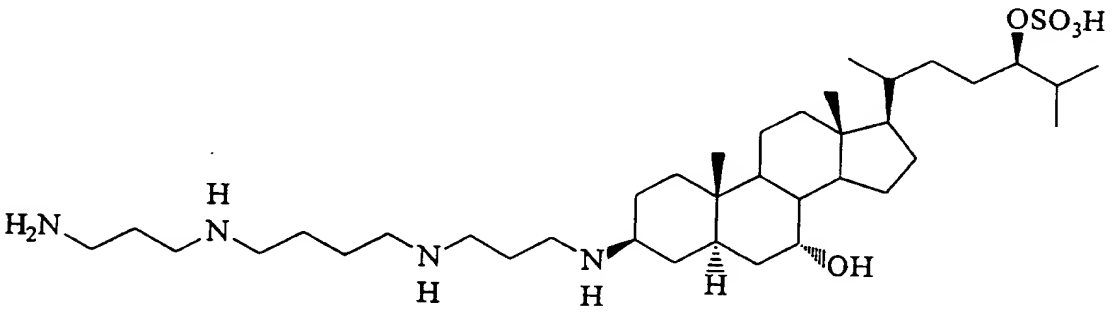
a pharmaceutically acceptable salt of compound 1521.

17. A method according to claim 16, wherein the ulcer is a gastric ulcer.

18. A method according to claim 16, wherein the ulcer is a duodenal ulcer.

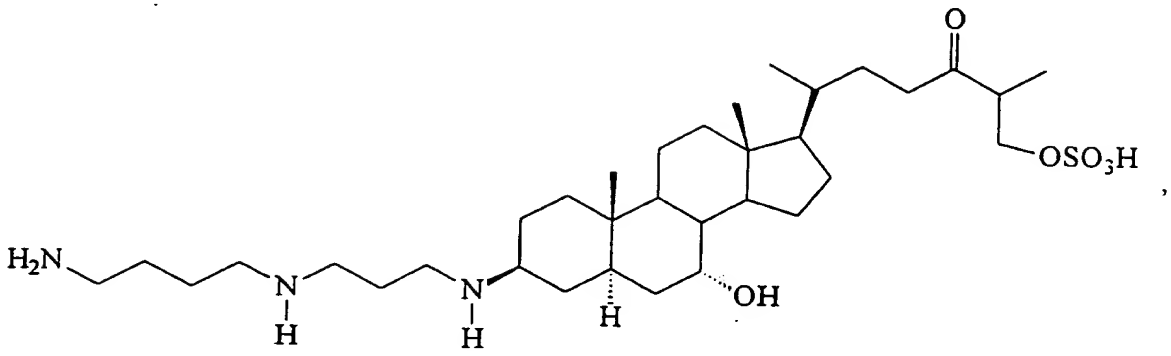
19. A method for treating a condition in a patient, wherein the condition is selected from the group consisting of: cardiac arrhythmia, Sjögren's syndrome, head trauma, constipation, and post-menopausal dry mucosa syndrome, the method comprising:

administering to the patient an effective amount of a pharmaceutical composition, wherein the pharmaceutical composition includes a pharmaceutically acceptable carrier or excipient, and a compound selected from the group consisting of: a compound according to formula 1436 as follows:

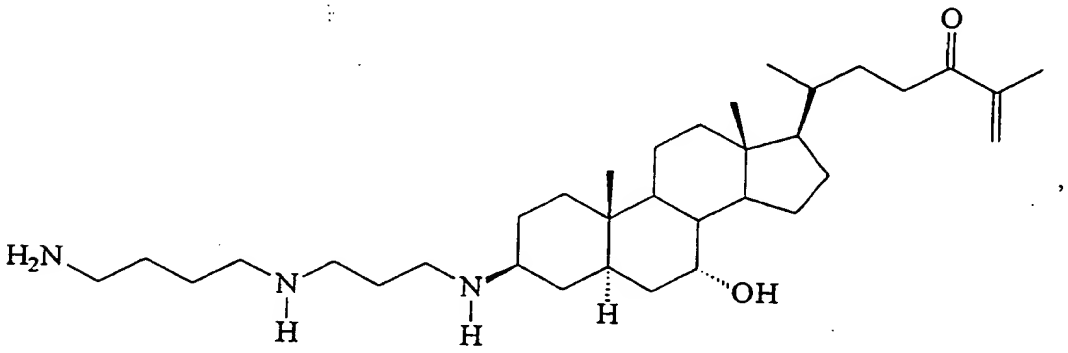


,

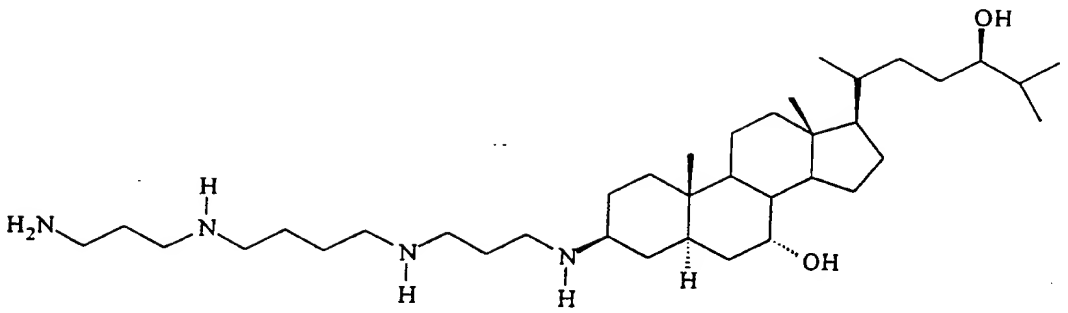
a pharmaceutically acceptable salt of compound 1436, a compound according to formula 1360A as follows:



a pharmaceutically acceptable salt of compound 1360A, a compound according to formula 1361A as follows:



a pharmaceutically acceptable salt of compound 1361A, a compound according to formula 1521 as follows:

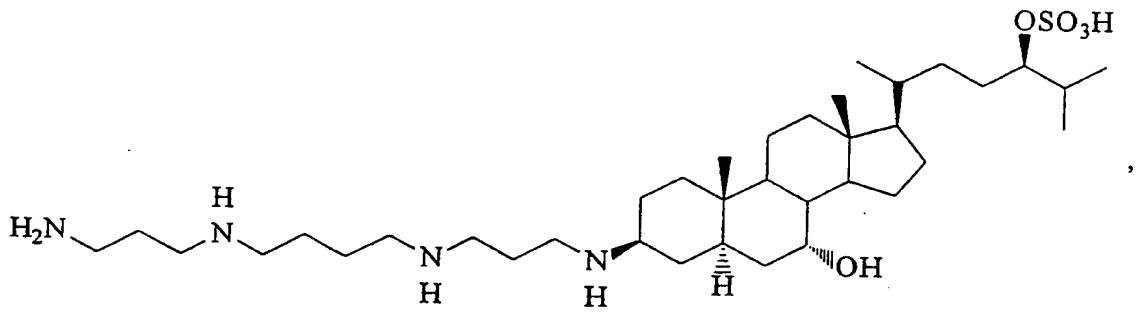


, and

a pharmaceutically acceptable salt of compound 1521.

20. A method for treating a condition in a patient, wherein the condition is selected from the group consisting of: cystic fibrosis, osteoporosis, and hypertension, the method comprising:

administering to the patient an effective amount of a pharmaceutical composition, wherein the pharmaceutical composition includes a pharmaceutically acceptable carrier or excipient, and a compound selected from the group consisting of: a compound according to formula 1436 as follows:



,

a pharmaceutically acceptable salt of compound 1436.

COMPOUND-1436

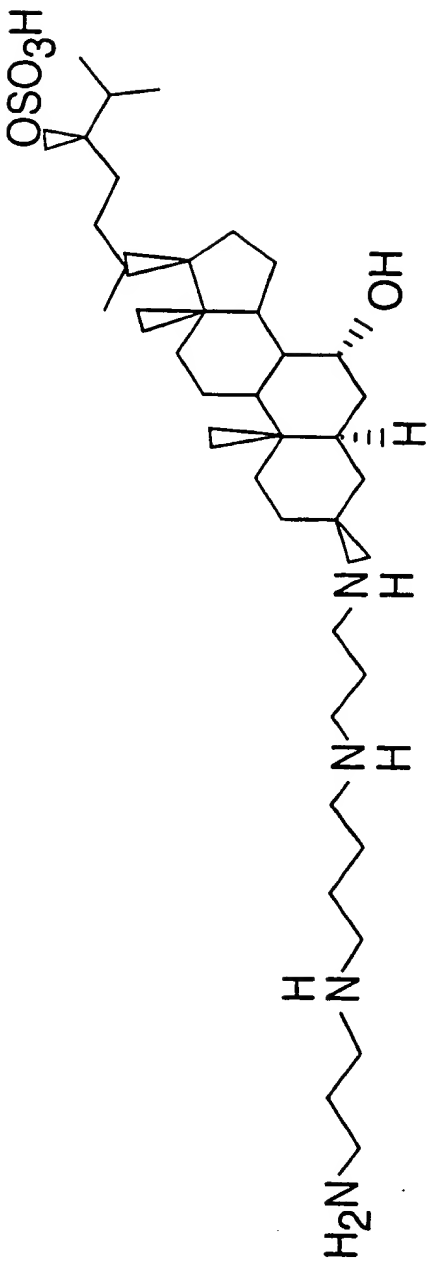
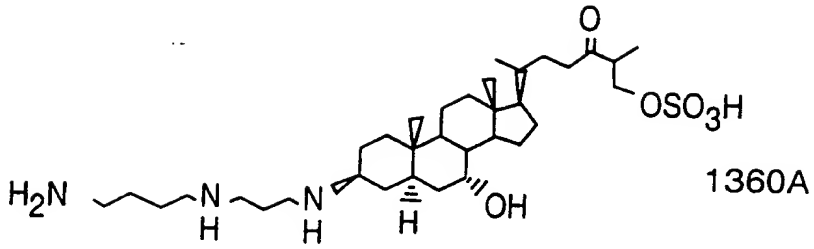
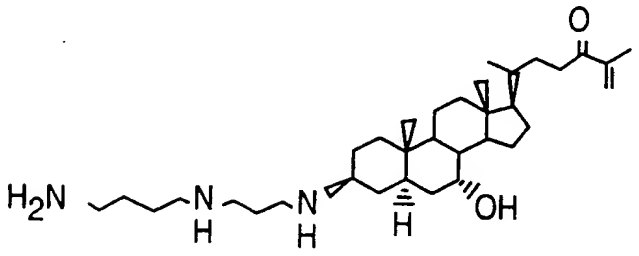


FIG. 1A



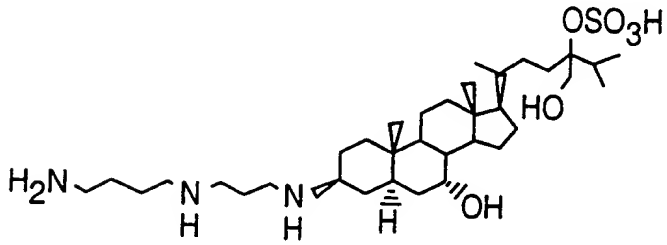
1360A

FIG. 1B



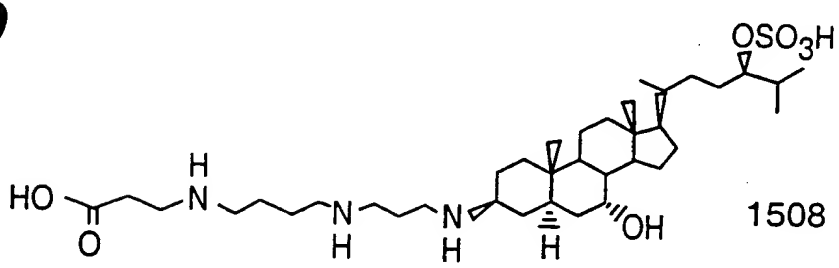
1361A

FIG. 1C



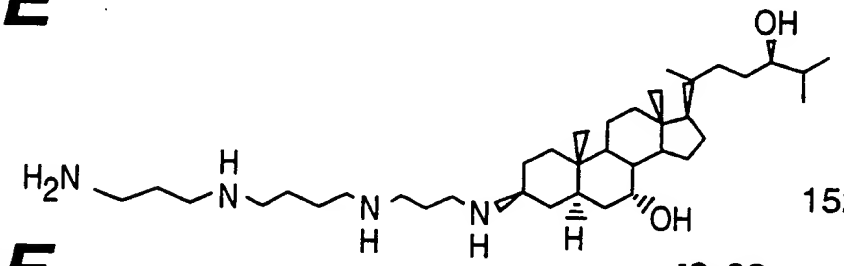
1437

FIG. 1D



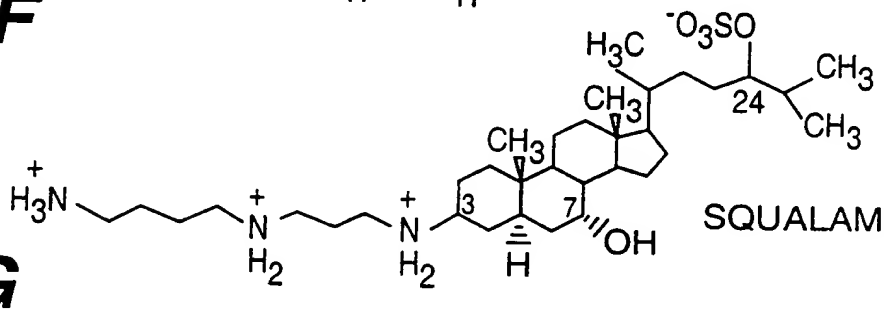
1508

FIG. 1E



1521

FIG. 1F



SQUALAMINE

FIG. 1G

EFFECT OF 1436 ON $^{36}Cl^-$ EFFLUX AND INFLUX IN XENOPUS OOCYTES

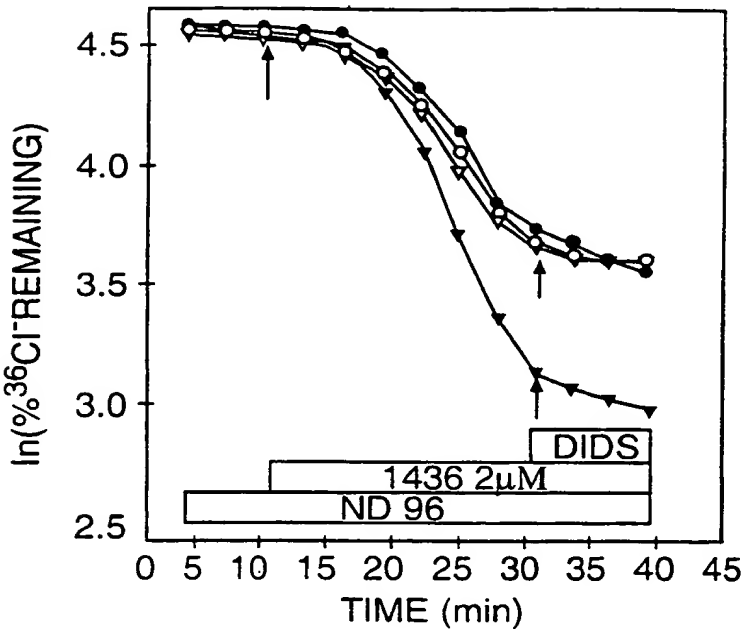


FIG. 2a

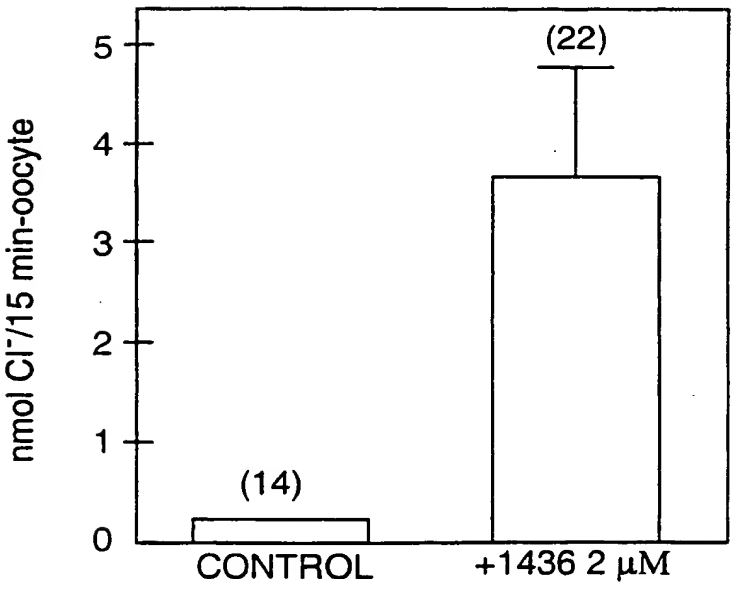


FIG. 2b

1436 ACTIVATES Cl EFFLUX WITHOUT TRANS-ANION REQUIREMENT

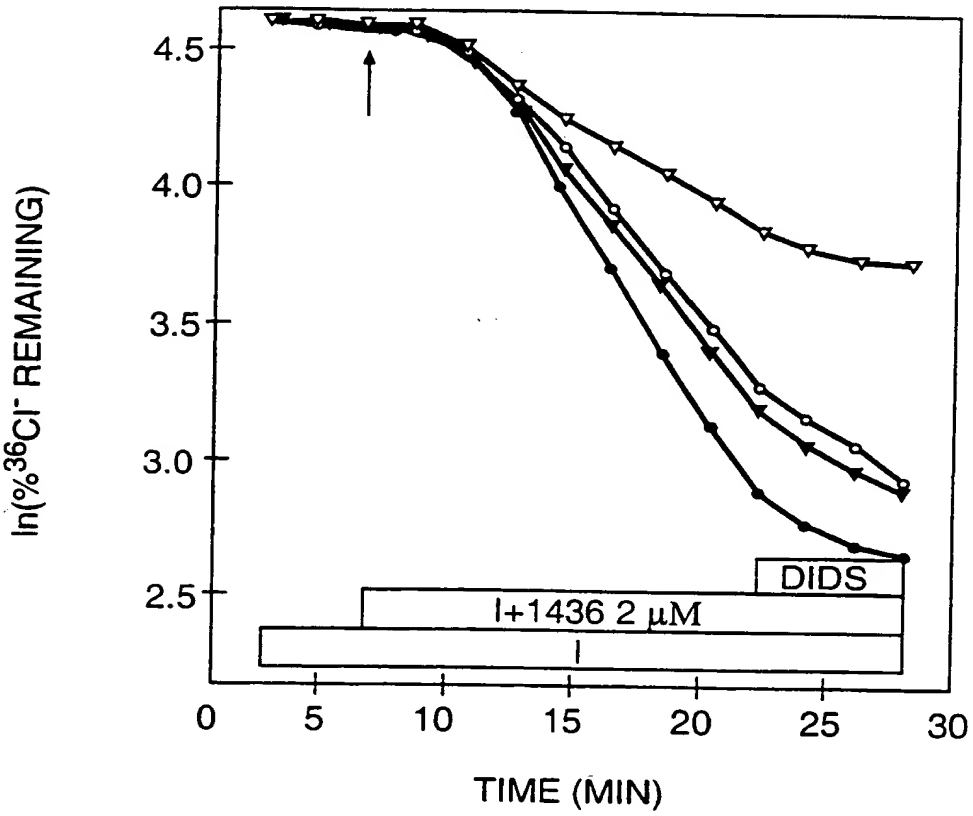


FIG. 3

1436 IS INACTIVE WHEN INJECTED INTO OOCYTES

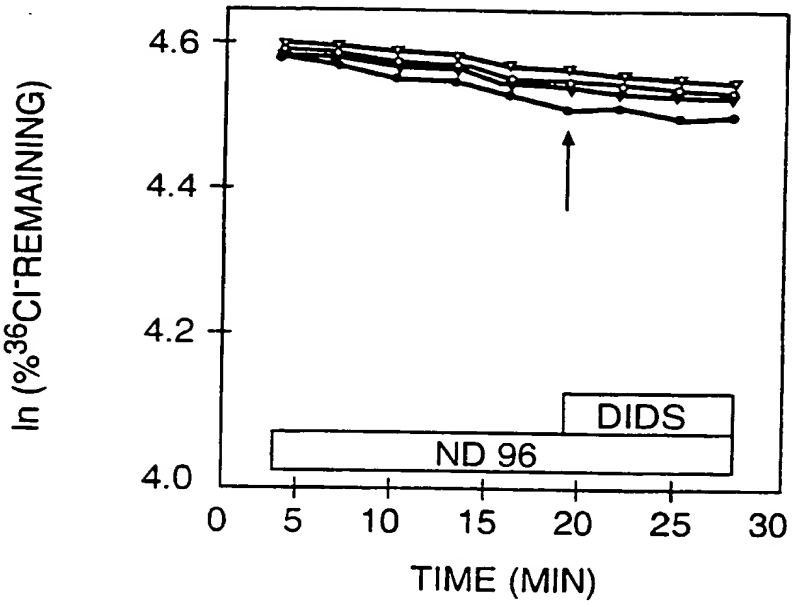


FIG. 4A

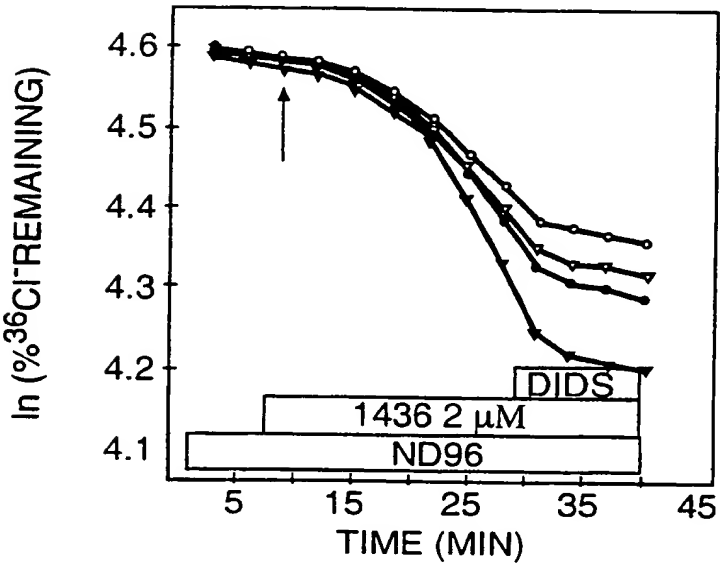


FIG. 4B

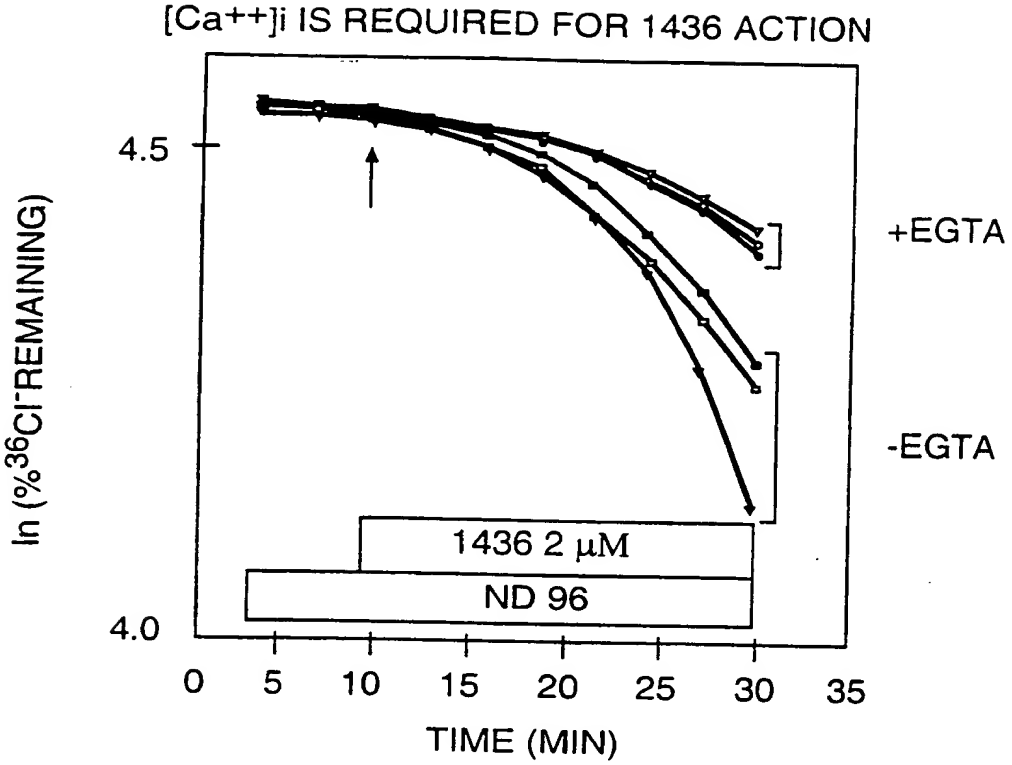

[Ca++]i IS REQUIRED FOR 1436 ACTION
4.5
4.0
ln (%36Cl-REMAINING)
+EGTA
-EGTA
1436 2 µM
ND 96
0
5
10
15
20
25
30
35
TIME (MIN)

FIG. 5A

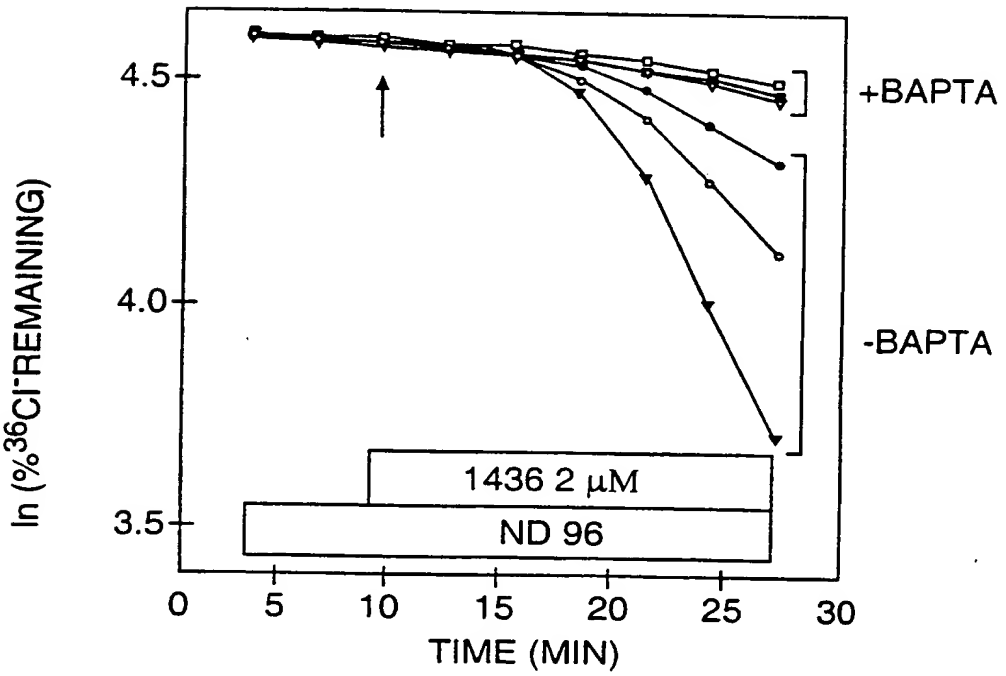

4.5
4.0
3.5
ln (%36Cl-REMAINING)
+BAPTA
-BAPTA
1436 2 µM
ND 96
0
5
10
15
20
25
30
TIME (MIN)

FIG. 5B

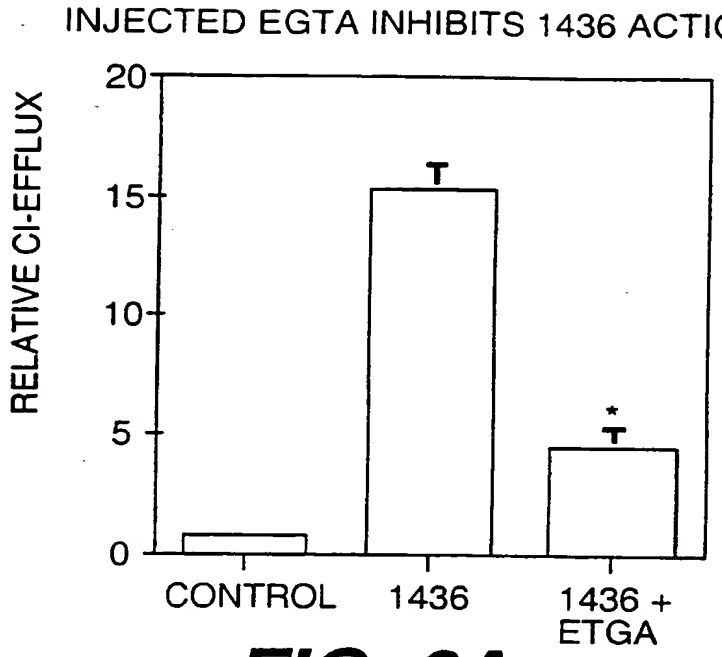

INJECTED EGTA INHIBITS 1436 ACTION
RELATIVE Cl-EFFLUX
20
15
10
5
0
CONTROL
1436
1436 + ETGA

FIG. 6A

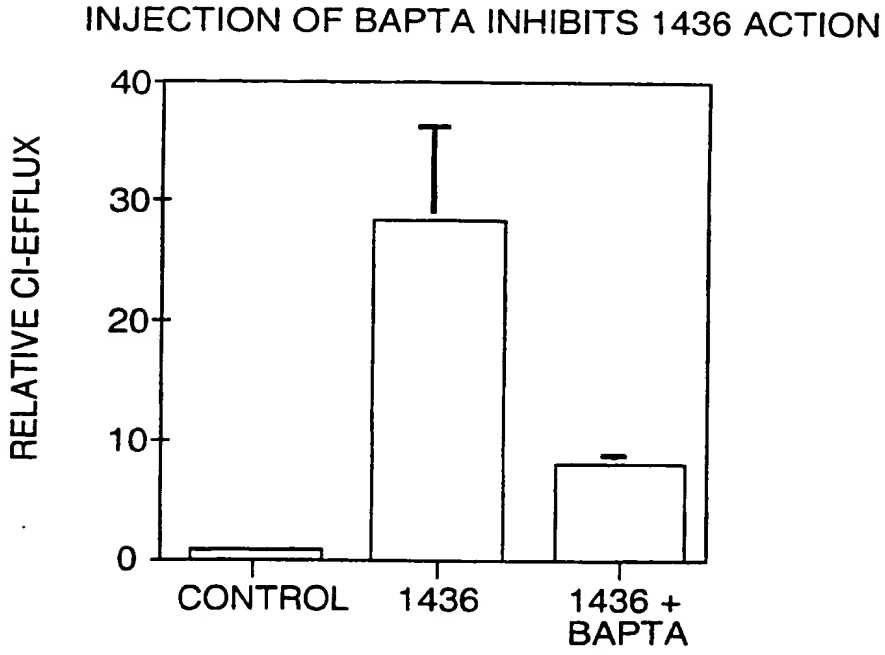

INJECTION OF BAPTA INHIBITS 1436 ACTION
RELATIVE Cl-EFFLUX
40
30
20
10
0
CONTROL
1436
1436 + BAPTA

FIG. 6B

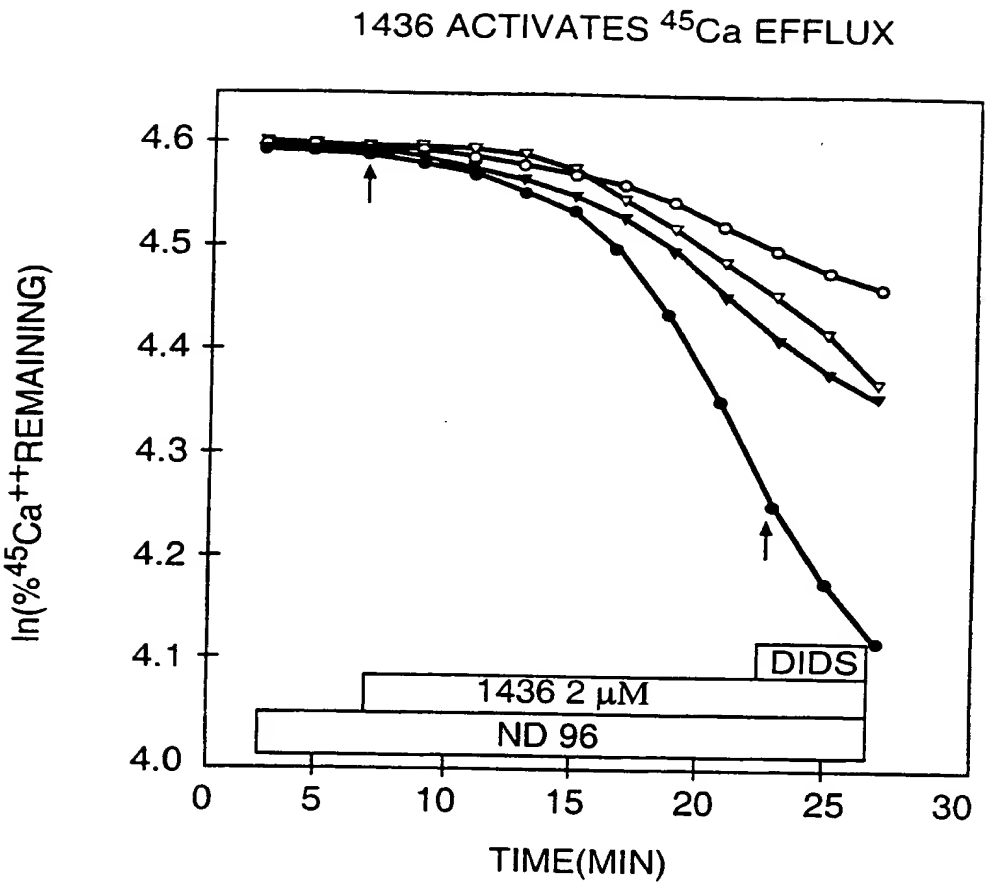

1436 ACTIVATES ^{45}Ca EFFLUX
ln(%$^{45}Ca^{++}$REMAINING)
4.6
4.5
4.4
4.3
4.2
4.1
4.0
0
5
10
15
20
25
30
TIME(MIN)
DIDS
1436 2 μM
ND 96

FIG. 7

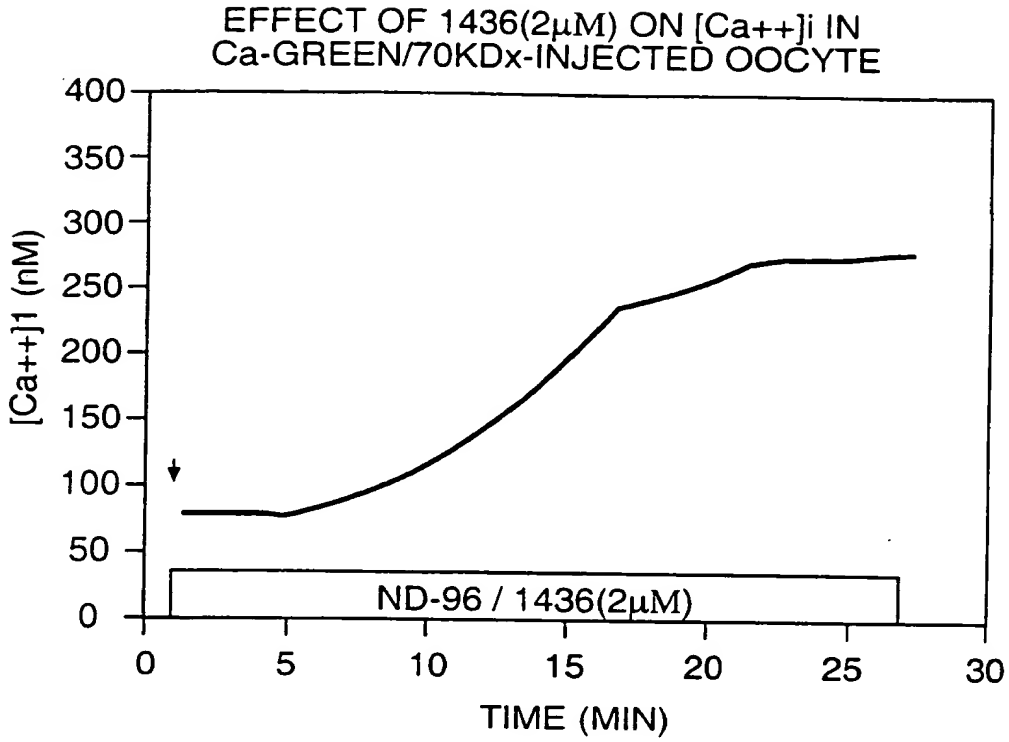

EFFECT OF 1436(2μM) ON [Ca++]i IN
Ca-GREEN/70KDx-INJECTED OOCYTE
[Ca++]1 (nM)
400
350
300
250
200
150
100
50
0
ND-96 / 1436(2μM)
0
5
10
15
20
25
30
TIME (MIN)

FIG. 8A

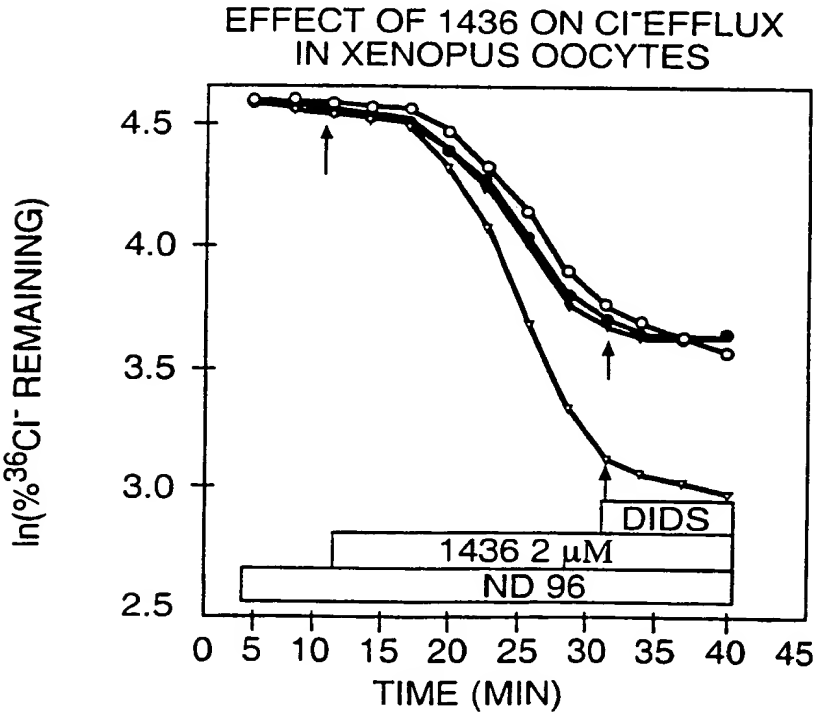

EFFECT OF 1436 ON Cl⁻EFFLUX
IN XENOPUS OOCYTES
ln(%36Cl⁻ REMAINING)
4.5
4.0
3.5
3.0
2.5
DIDS
1436 2 μM
ND 96
0 5 10 15 20 25 30 35 40 45
TIME (MIN)

FIG. 8B

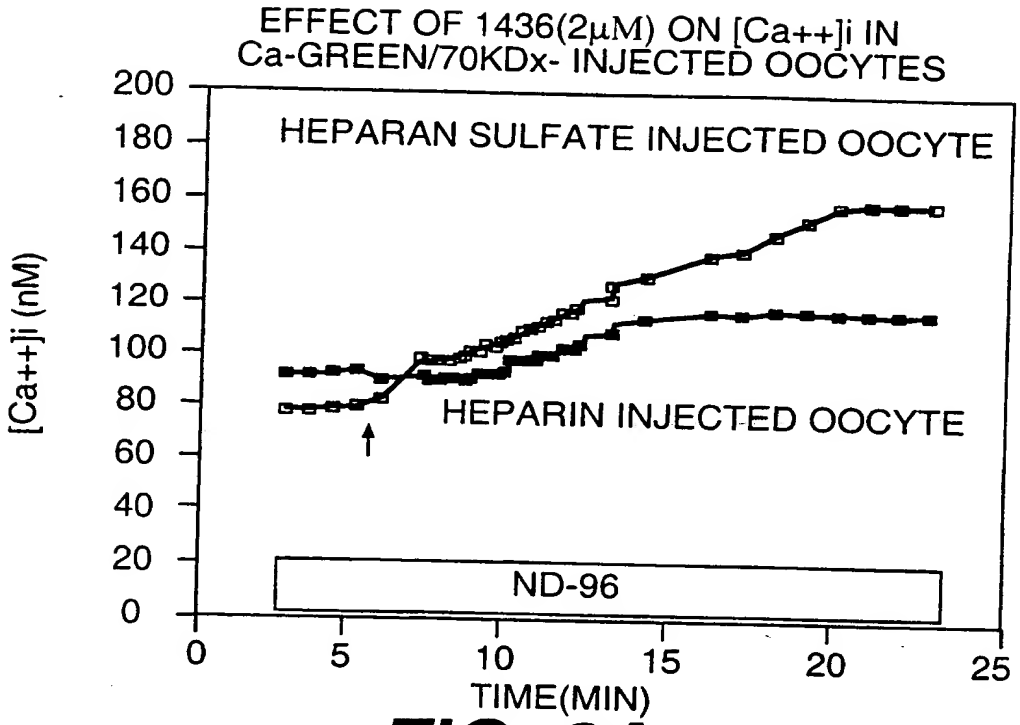

EFFECT OF 1436(2μM) ON [Ca++]i IN
Ca-GREEN/70KDx- INJECTED OOCYTES
HEPARAN SULFATE INJECTED OOCYTE
HEPARIN INJECTED OOCYTE
ND-96
[Ca++]i (nM)
TIME(MIN)
0
20
40
60
80
100
120
140
160
180
200
0
5
10
15
20
25

FIG. 9A

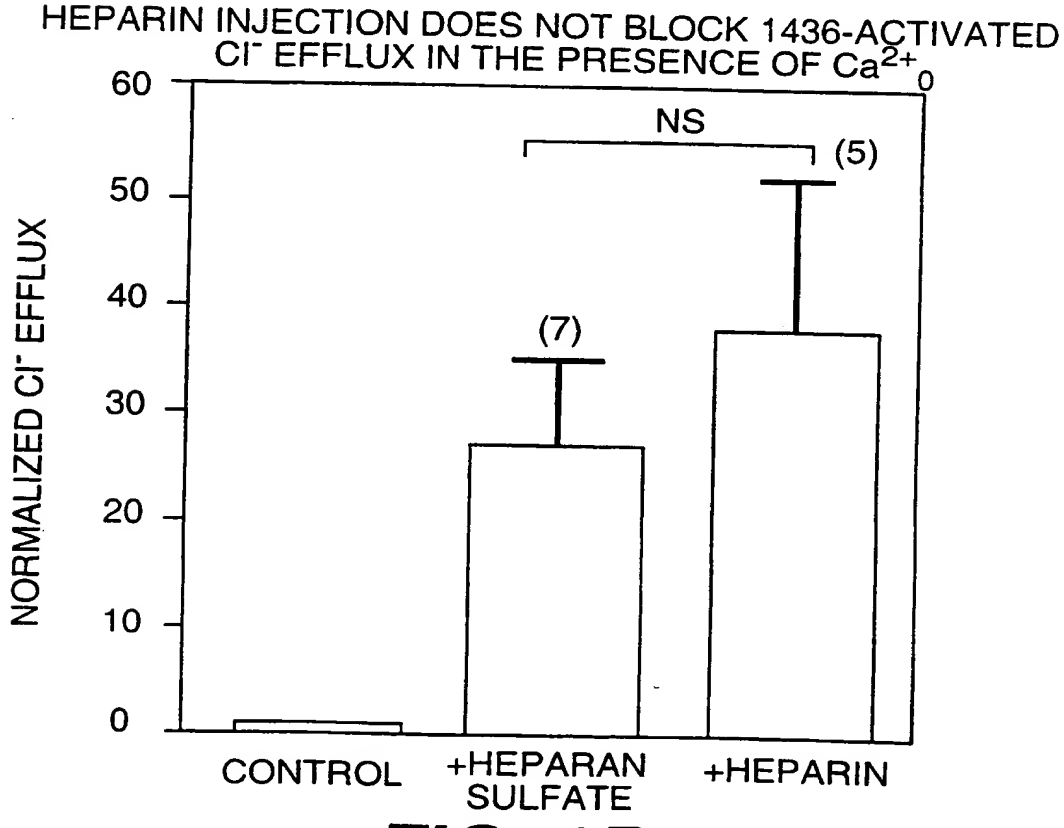

HEPARIN INJECTION DOES NOT BLOCK 1436-ACTIVATED
Cl⁻ EFFLUX IN THE PRESENCE OF Ca2+0
NS
(5)
(7)
NORMALIZED Cl⁻ EFFLUX
0
10
20
30
40
50
60
CONTROL
+HEPARAN SULFATE
+HEPARIN

FIG. 9B

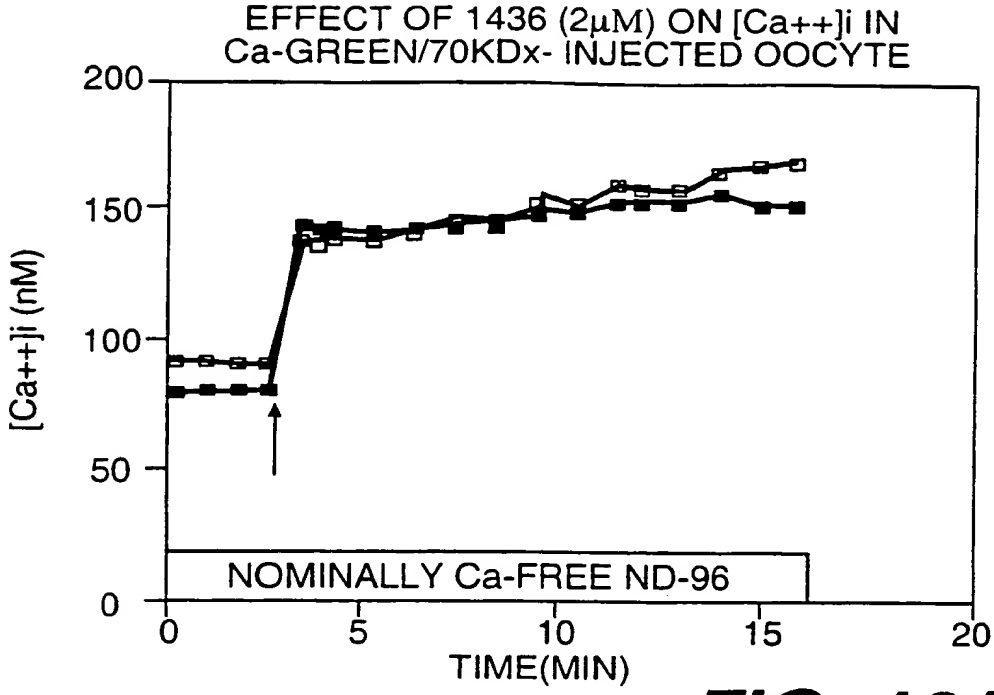


FIG. 10A

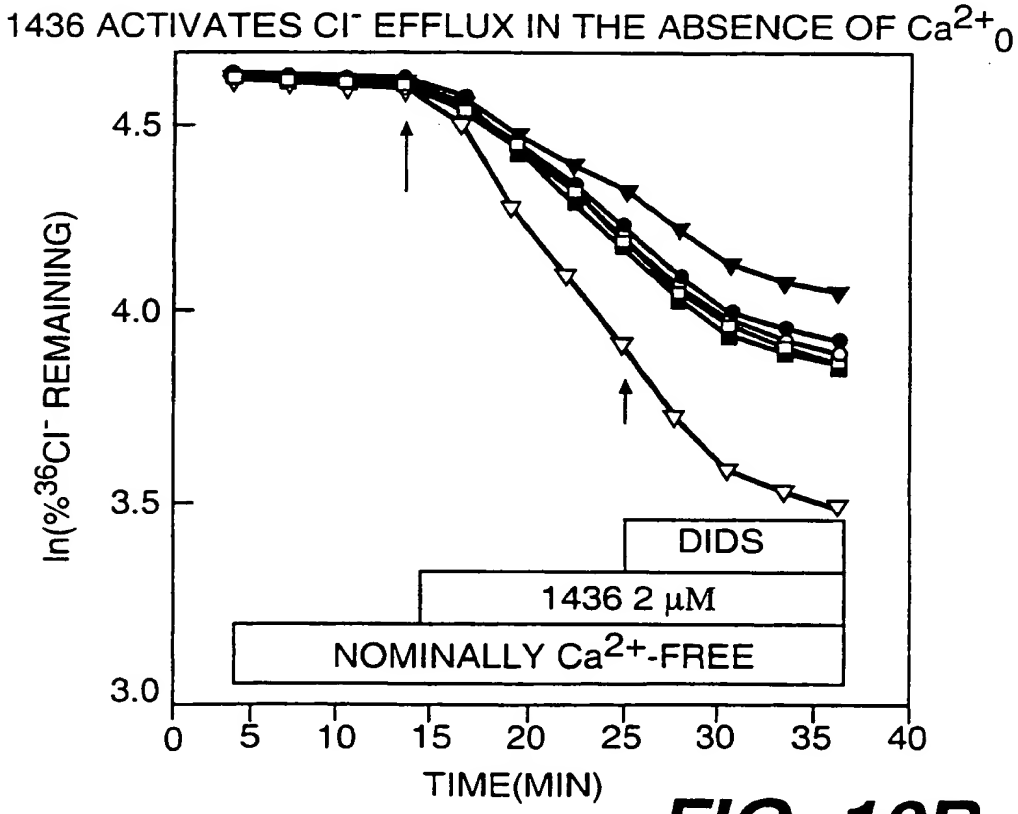


FIG. 10B

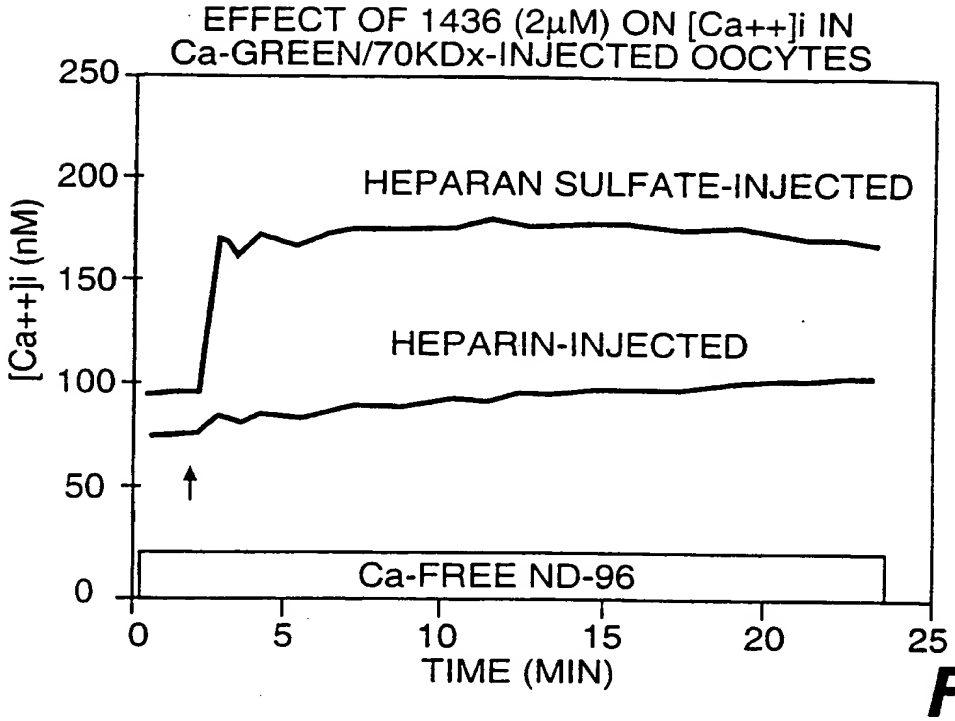


FIG. 11A

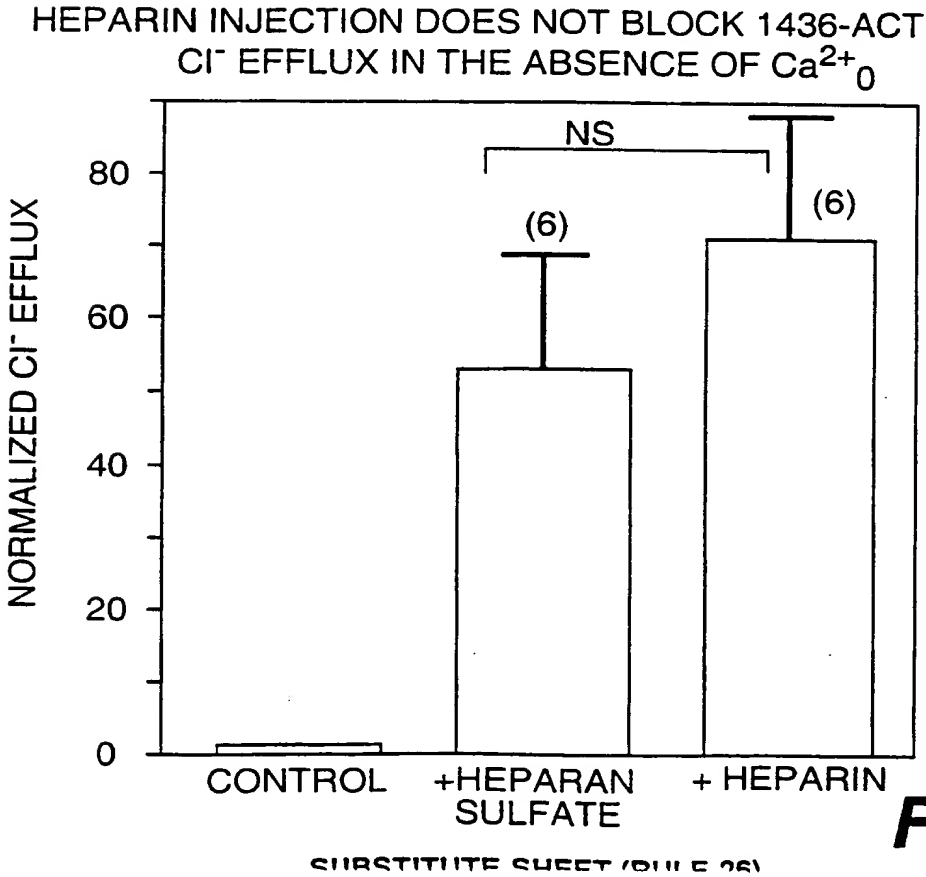


FIG. 11B

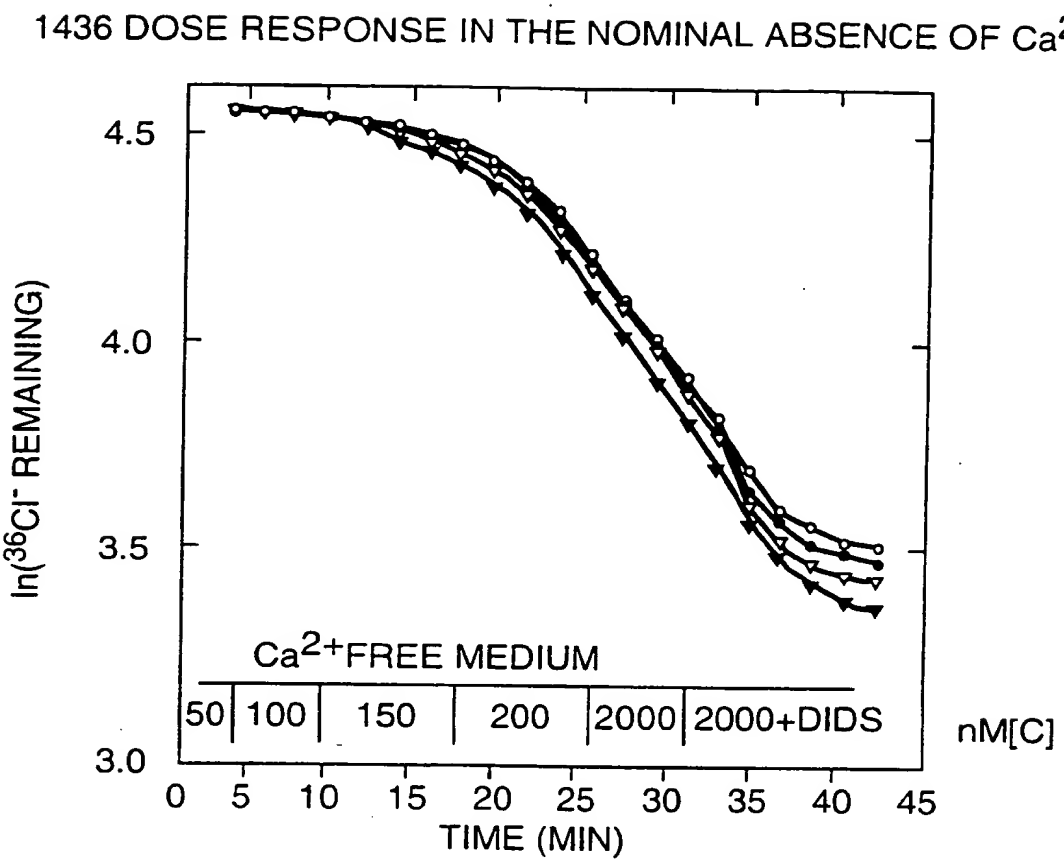

1436 DOSE RESPONSE IN THE NOMINAL ABSENCE OF Ca^{2+}_0
4.5
4.0
3.5
3.0
$ln(^{36}Cl^-$ REMAINING)
Ca^{2+}FREE MEDIUM
50
100
150
200
2000
2000+DIDS
nM[C]
0
5
10
15
20
25
30
35
40
45
TIME (MIN)

FIG. 12

EFFECTS ON OOCYTE Cl^- EFFLUX OF 2μM 1436 IN:

	ND 96	NOMINALLY Ca^{2+}-FREE ND-96
1360	INACTIVE	WEAKLY ACTIVE
1361	INACTIVE	INACTIVE
1436	ACTIVE	ACTIVE
1437	INACTIVE	ACTIVE
1508	INACTIVE	MODERATELY ACTIVE
1521	INACTIVE	WEAKLY ACTIVE
SQUALAMINE	INACTIVE	ACTIVE

FIG. 13

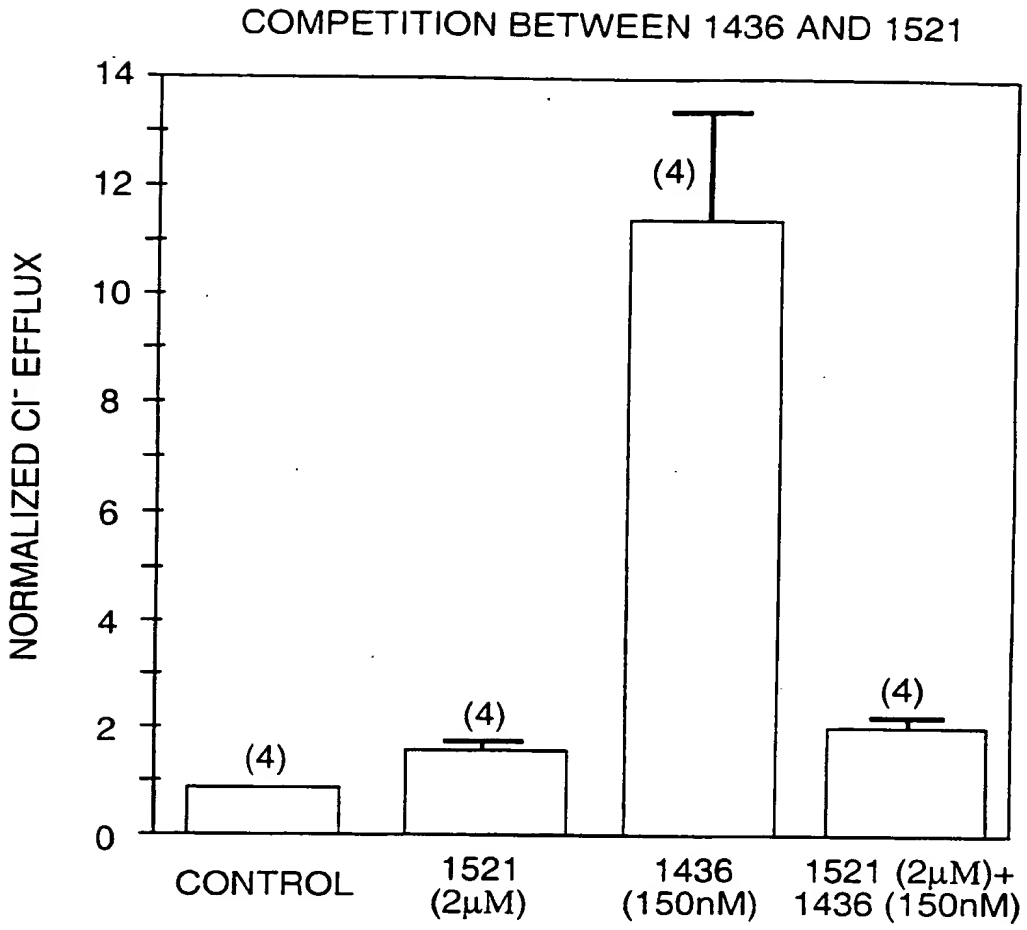

COMPETITION BETWEEN 1436 AND 1521
NORMALIZED Cl⁻ EFFLUX
14
12
10
8
6
4
2
0
(4)
(4)
(4)
(4)
CONTROL
1521 (2μM)
1436 (150nM)
1521 (2μM)+ 1436 (150nM)

FIG. 14

IN THE PRESENCE OF 1521, DIDS ACTIVATES Cl^- EFFLUX

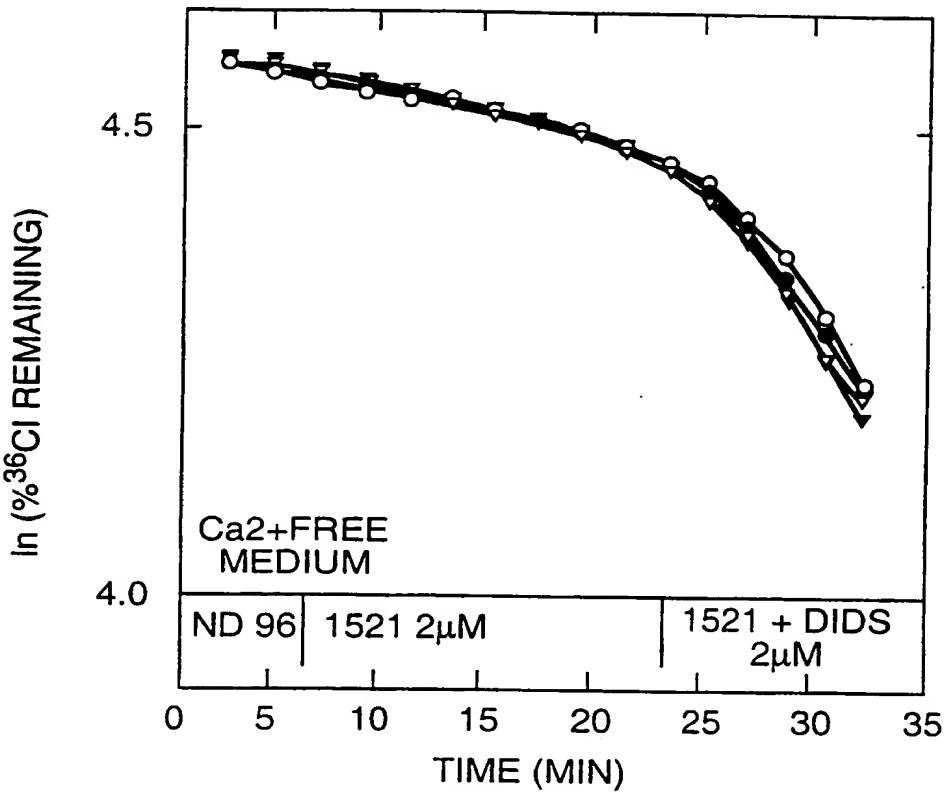


FIG. 15

EFFECTS OF GUANINE NUCLEOTIDES ON 1436 ACTION

EXPT. # (N=3)	-γ-S-GTP (FOLD STIMULATION OF $^{36}Cl^-$ EFFLUX BY 1436)	+γ-S-GTP	
1. (100μM)	13.5 ± 2.9	10.6 ± 3.1	0.79
2. (100μM)	35.7 ± 6.3	11.9 ± 5.6*	0.33
3. (100μM)	14.1 ± 4.3	4.9 ± 0.6*	0.35
4. (500μM) Ca^{2+}-FREE	8.5 ± 1.3	4.9 ± 0.5	0.58 (0.51 ± 0.11)

EXPT. # (N=3-5)	-β-S-GDP (FOLD STIMULATION OF $^{36}Cl^-$ EFFLUX BY 1436)	+β-S-GDP
1. (100μM)	14.3 ± 4.5	12.5 ± 1.4
2. (100μM) Ca^{2+}-FREE	7.6 ± 0.5	8.5 ± 1.5
3. (500μM) Ca^{2+}-FREE	10.0 ± 0.8	1.8 ± 0.1*
4. (500μM) Ca^{2+}-FREE	10.0 ± 2.5	10.9 ± 2.9

FIG. 16

INHIBITION OF AE1 AND AE2 BY SQUALAMINE DERIVATIVES

MAGAININ # 2 (μM)	% RESIDUAL ACTIVITY (±sem,n)	
	AE1	AE2
1361A	72.1 ± 6.7% (7)	17.5 ± 2.7% (8)
1360A(1361 SULFATE)	85.3 ± 0.7% (3)	25.9 ±3.0% (2)
SQUALAMINE	93.5 ± 18.6% (4)	93.8 ± 14.2% (5)
1437 (HYDROXYSQUALAMINE)	96.6% (1)	57.1 ± 2.8% (3)
1521 (1436 DES-SULFATE)	77.0 ± 3.0% (2)	54.5 ± 12.4% (3)
1508 (1436 PAO METAB)	58.5 ± 7.5% (2)	78.2 ± 9.0% (3)
1436	CAN'T BE EVALUATED BY $^{36}Cl^-$FLUX	

FIG. 17

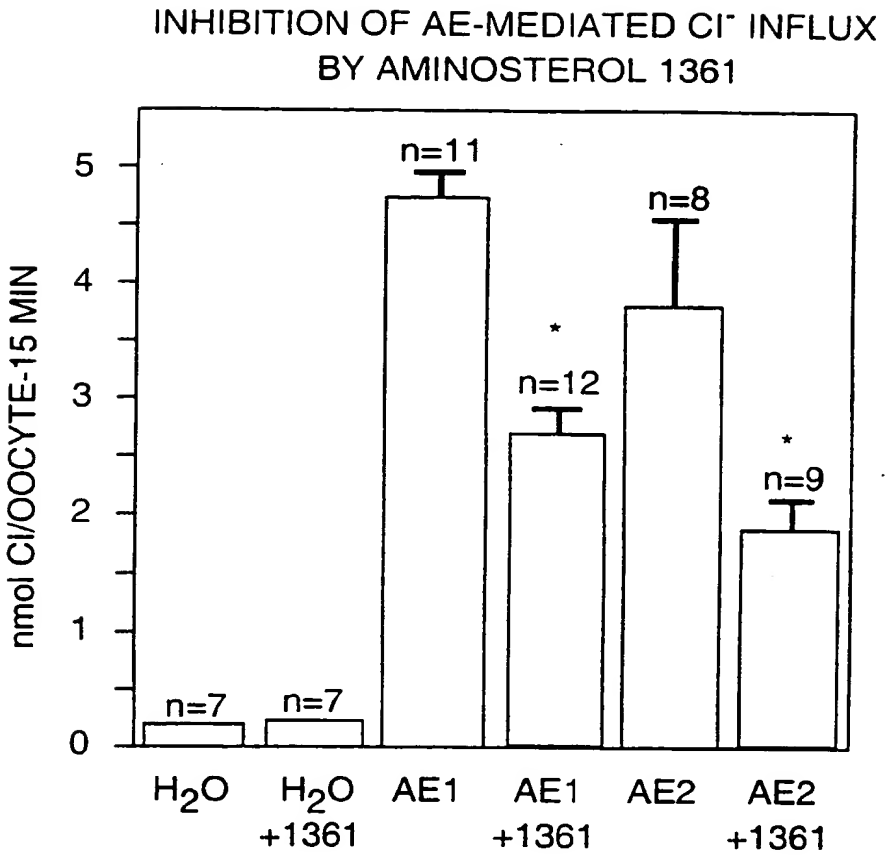

INHIBITION OF AE-MEDIATED Cl⁻ INFLUX
BY AMINOSTEROL 1361
nmol Cl/OOCYTE-15 MIN
0
1
2
3
4
5
n=7
n=7
n=11
n=12
n=8
n=9
*
*
H2O
H2O +1361
AE1
AE1 +1361
AE2
AE2 +1361

FIG. 18

REPRESENTATIVE DOSE-RESPONSE FOR 1360 INHIBITION OF AE2

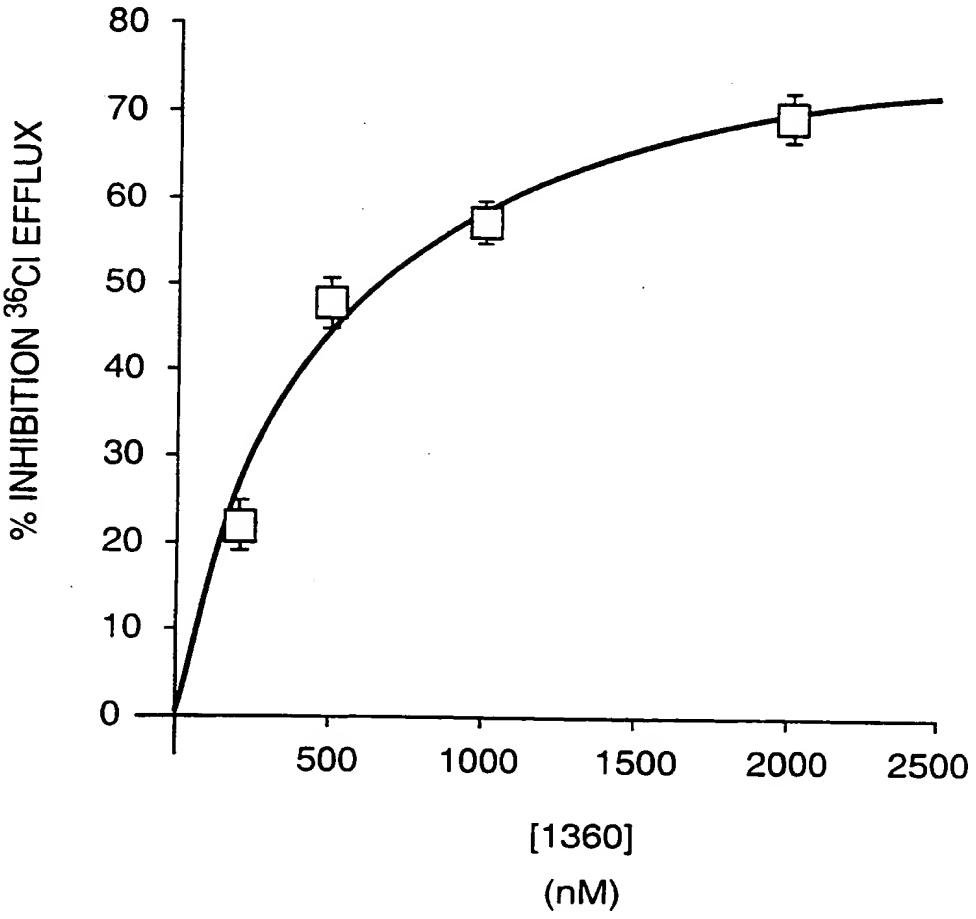


FIG. 19

ID_{50} VALUES OF AMINOSTEROLS FOR $^{36}Cl^-/Cl^-$ EXCHANGE MEDIATED BY AE1 AND AE2 IN XENOPUS OOCYTES

[nM±s.e.m. (# EXPERIMENTS)]

AMINOSTEROL	AE1	AE2
1361	1140 ± 580 (3)	140 ± 30 (5)
1360	>5000 (2)	500 ± 140 (3)

FIG. 20

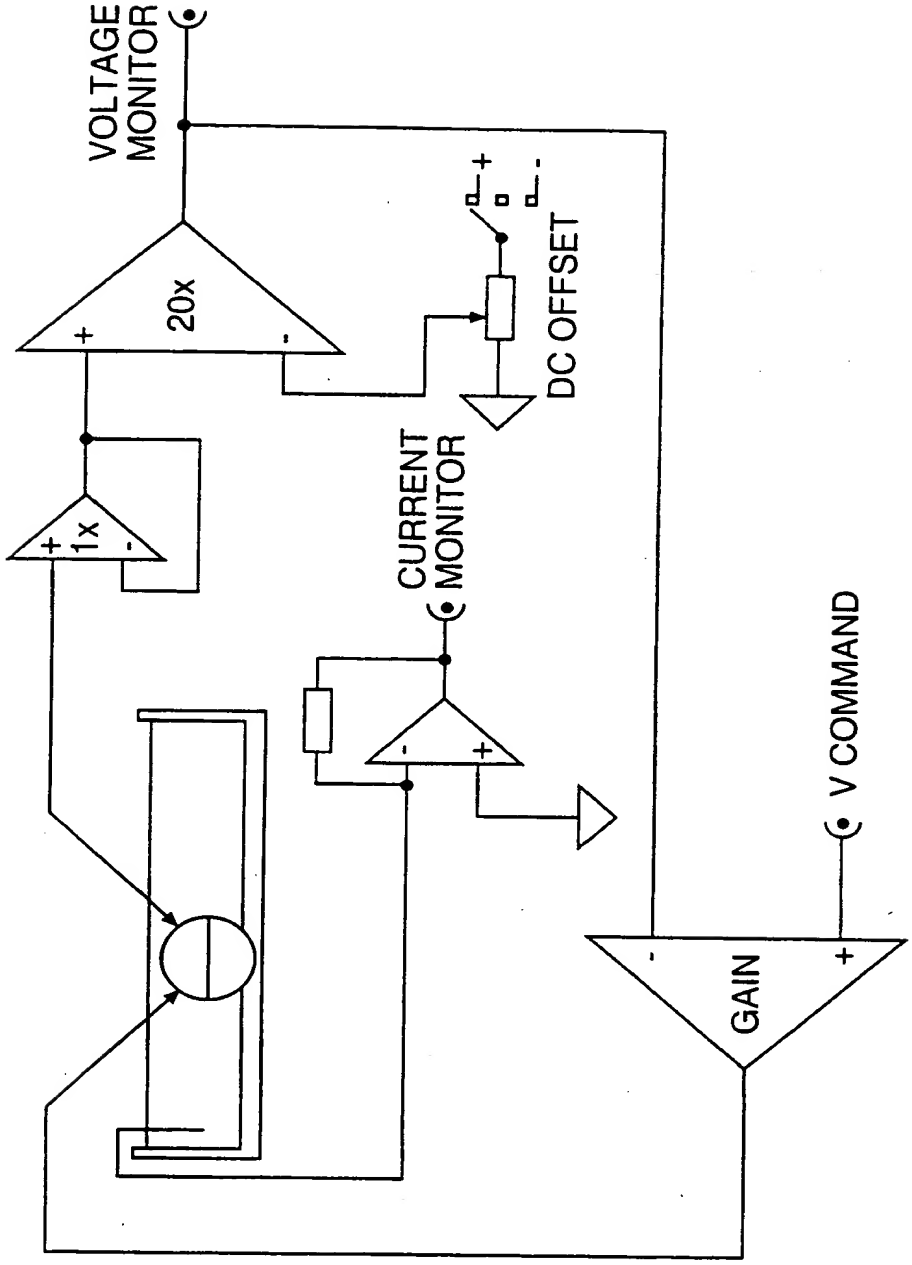

VOLTAGE MONITOR
20x
DC OFFSET
1x
CURRENT MONITOR
V COMMAND
GAIN

FIG. 21

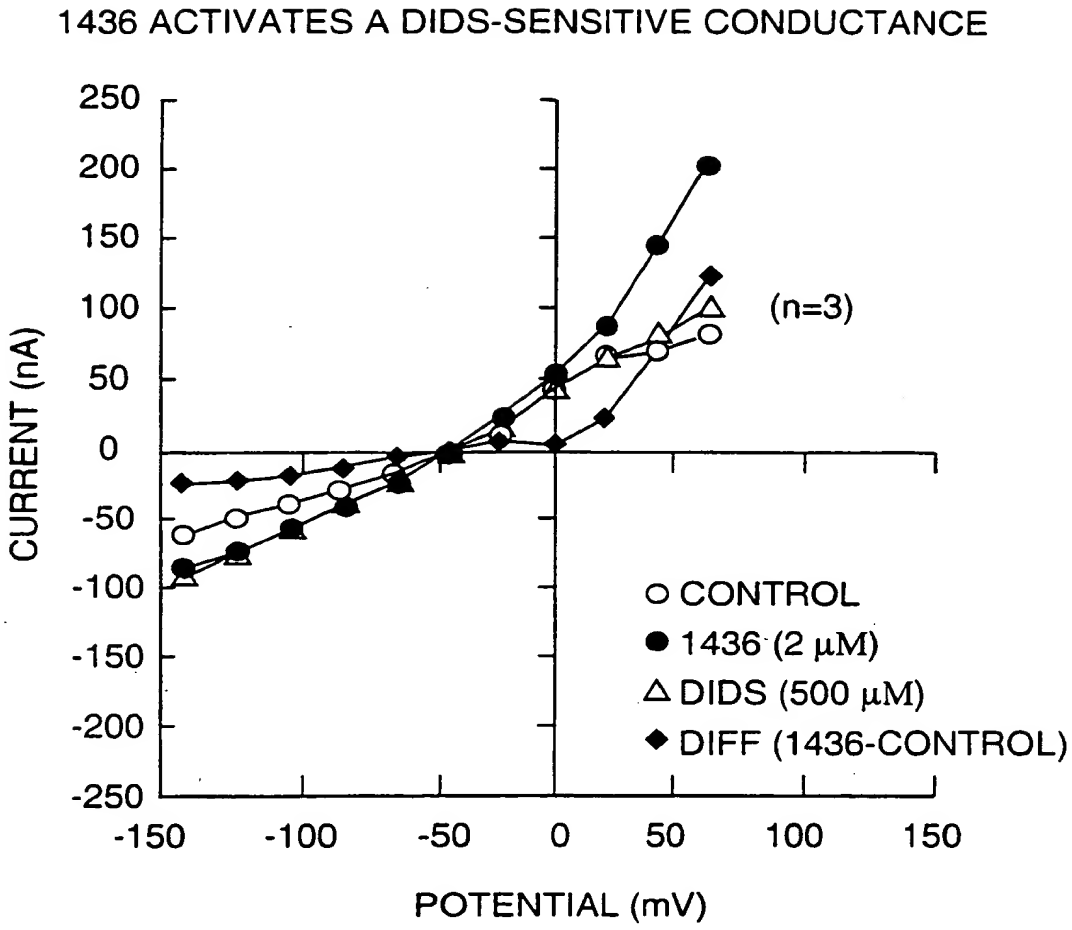

1436 ACTIVATES A DIDS-SENSITIVE CONDUCTANCE
250
200
150
100
50
0
-50
-100
-150
-200
-250
CURRENT (nA)
-150
-100
-50
0
50
100
150
POTENTIAL (mV)
(n=3)
CONTROL
1436 (2 μM)
DIDS (500 μM)
DIFF (1436-CONTROL)

FIG. 22

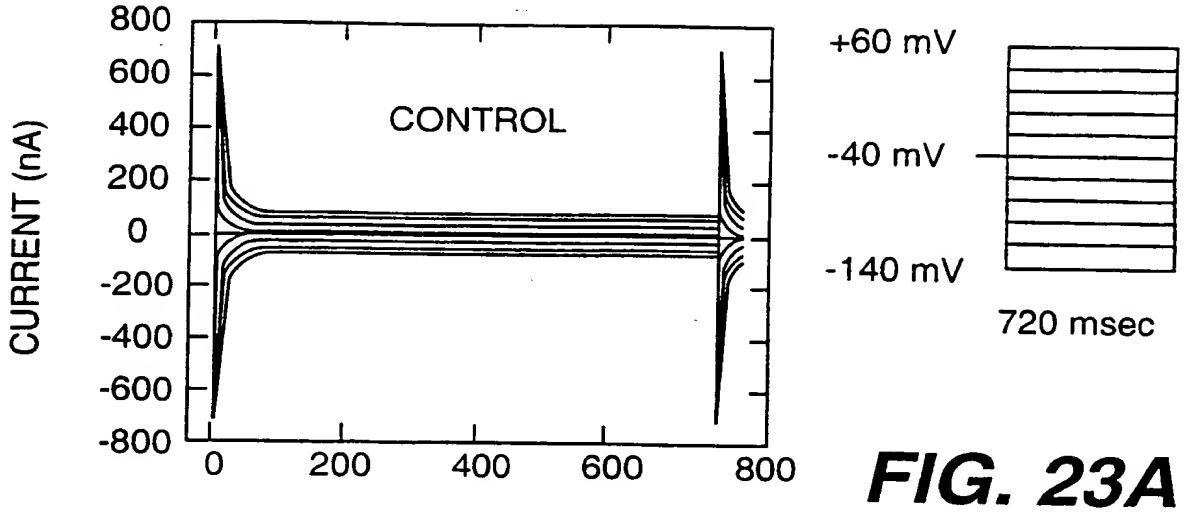


FIG. 23A

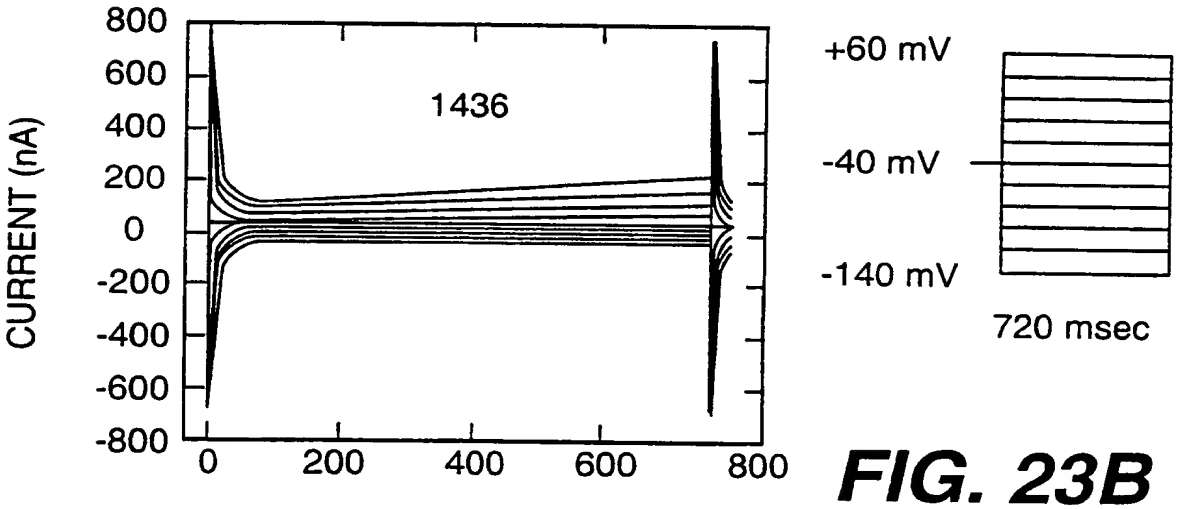


FIG. 23B

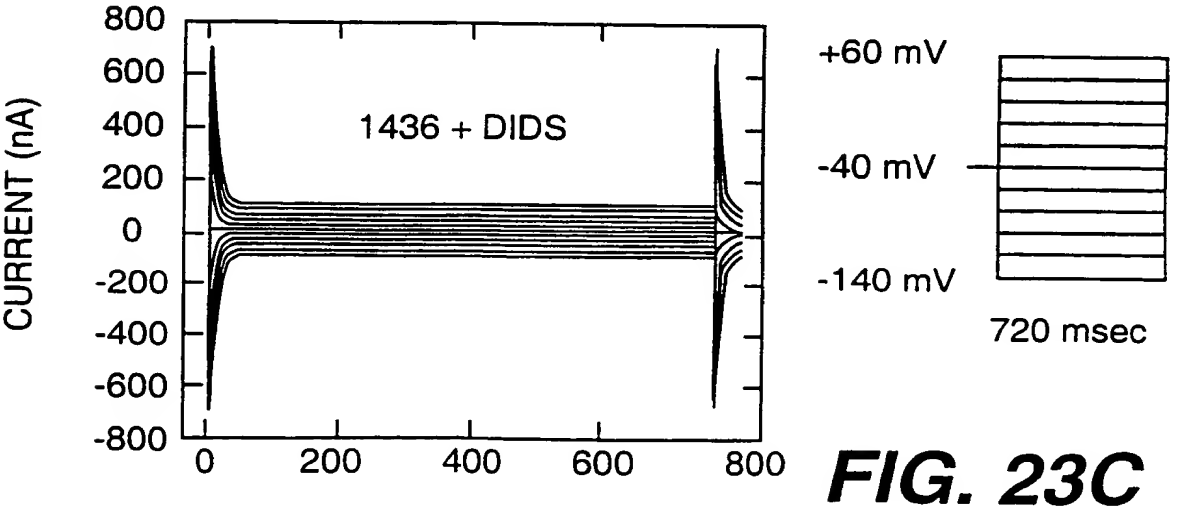


FIG. 23C

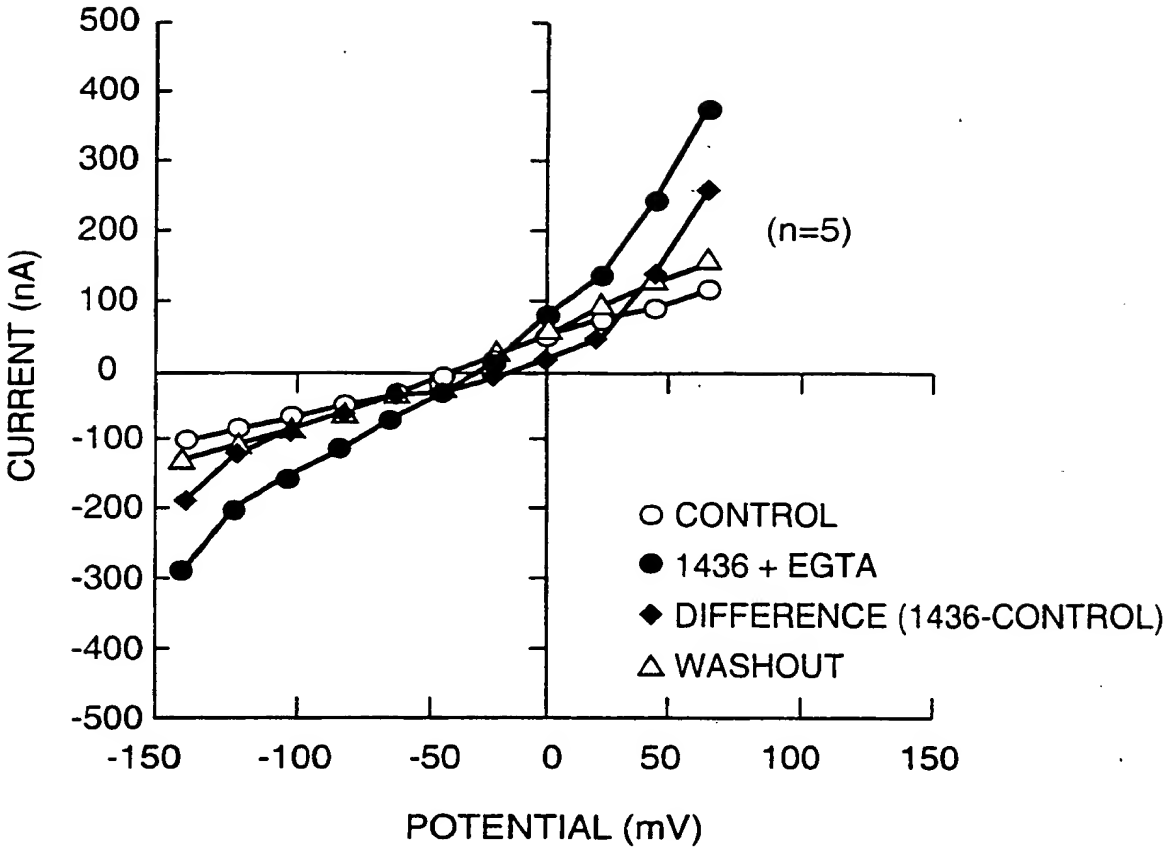

500
400
300
200
100
0
-100
-200
-300
-400
-500
CURRENT (nA)
-150
-100
-50
0
50
100
150
POTENTIAL (mV)
(n=5)
CONTROL
1436 + EGTA
DIFFERENCE (1436-CONTROL)
WASHOUT

FIG. 24

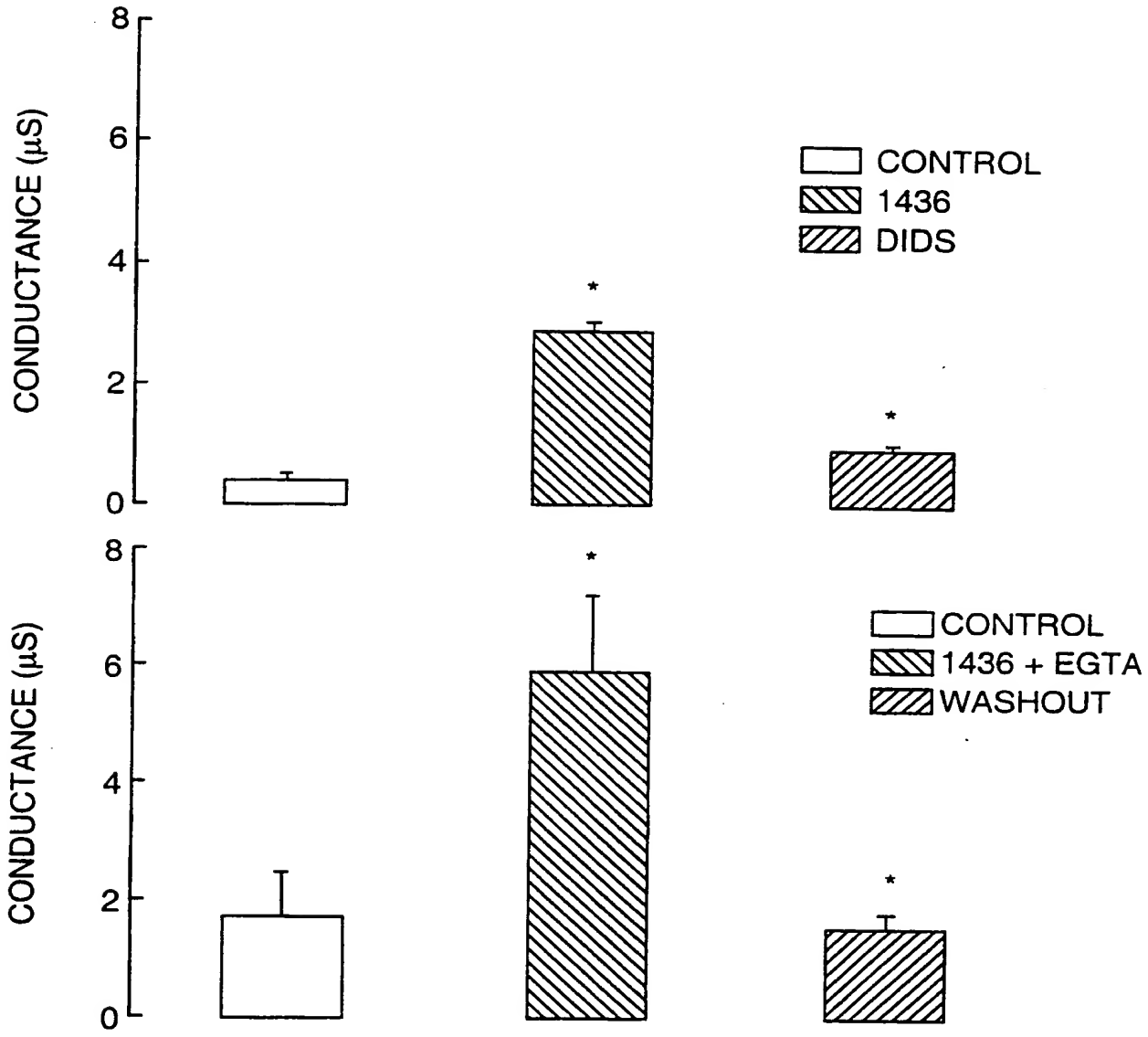

CONDUCTANCE (μS)
8
6
4
2
0
CONTROL
1436
DIDS
*
*
CONDUCTANCE (μS)
8
6
4
2
0
CONTROL
1436 + EGTA
WASHOUT
*
*

FIG. 25

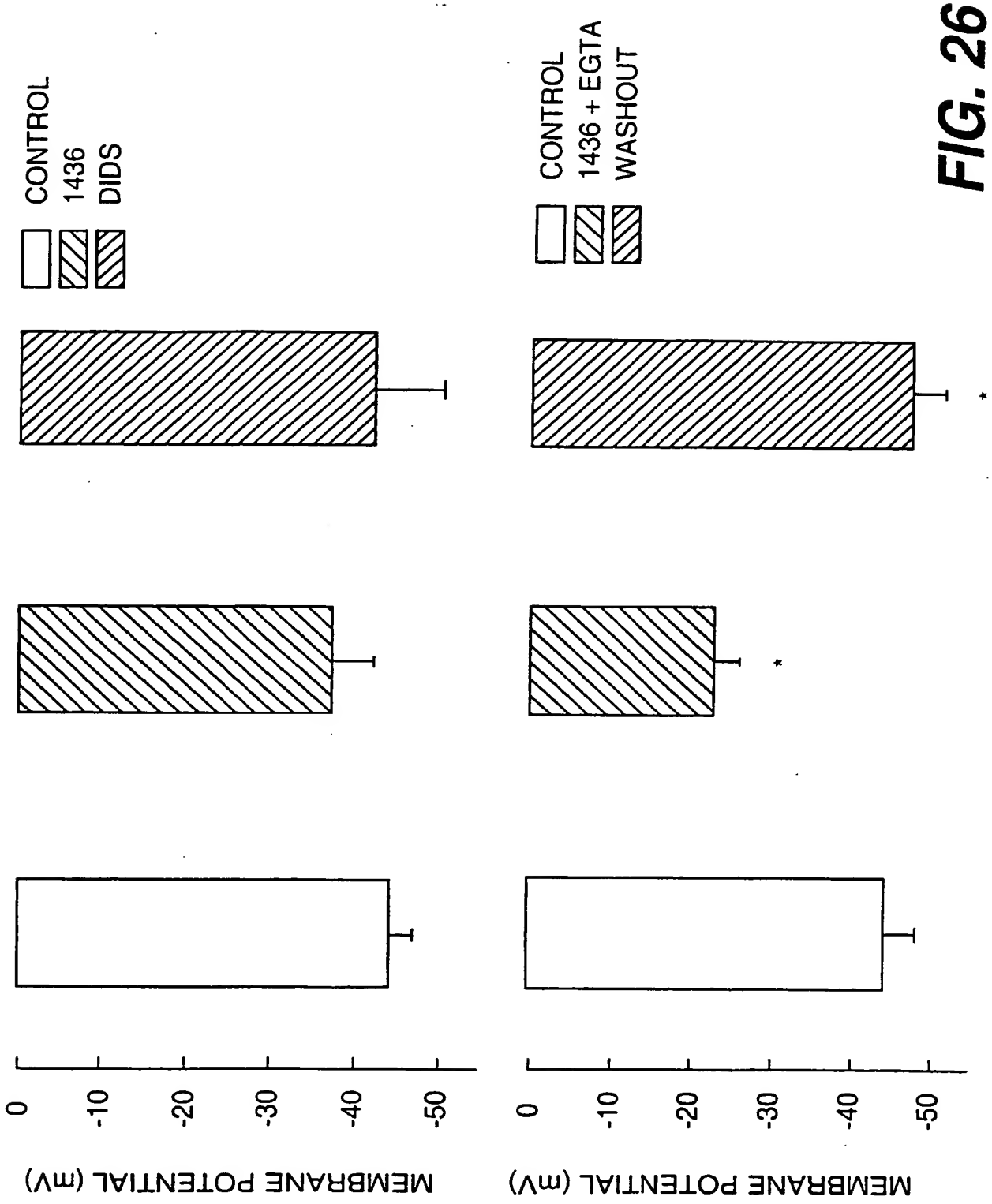

CONTROL
1436
DIDS
CONTROL
1436 + EGTA
WASHOUT
*
*
0
-10
-20
-30
-40
-50
MEMBRANE POTENTIAL (mV)
MEMBRANE POTENTIAL (mV)

FIG. 26

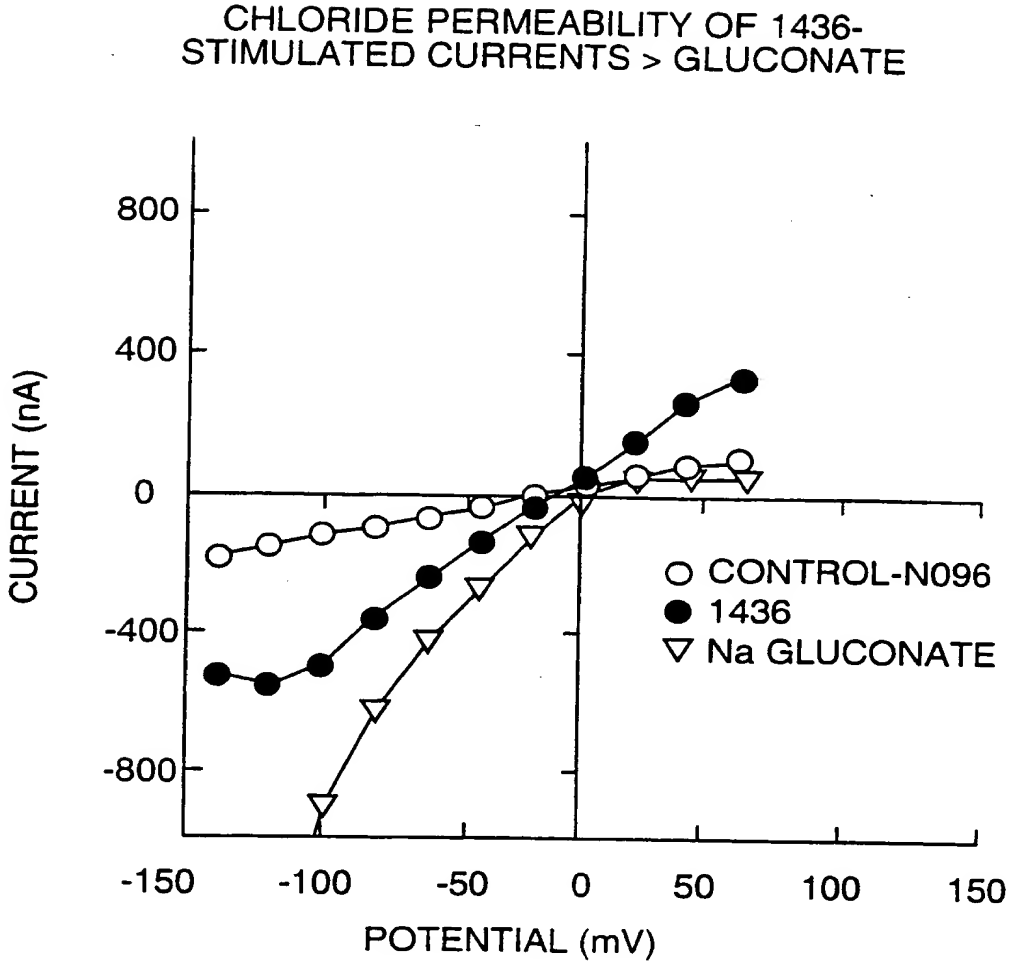

CHLORIDE PERMEABILITY OF 1436-
STIMULATED CURRENTS > GLUCONATE
800
400
0
-400
-800
CURRENT (nA)
-150
-100
-50
0
50
100
150
POTENTIAL (mV)
CONTROL-N096
1436
Na GLUCONATE

FIG. 27

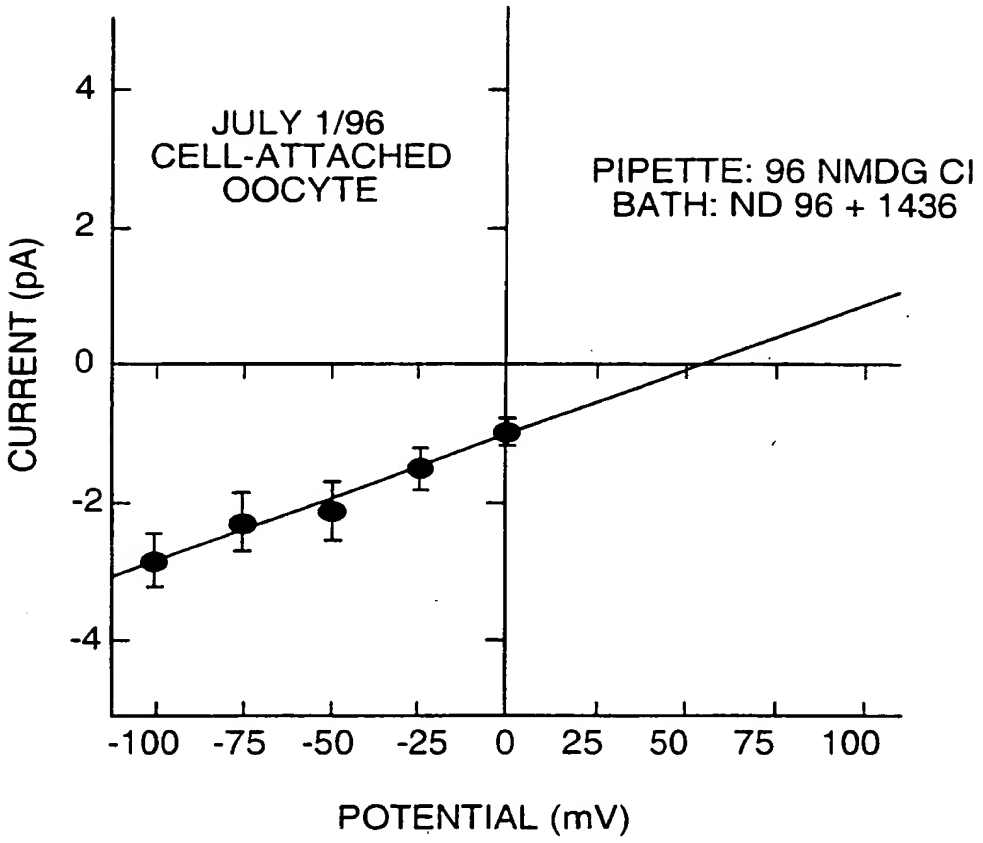

JULY 1/96
CELL-ATTACHED
OOCYTE
PIPETTE: 96 NMDG Cl
BATH: ND 96 + 1436
CURRENT (pA)
4
2
0
-2
-4
-100
-75
-50
-25
0
25
50
75
100
POTENTIAL (mV)

FIG. 28

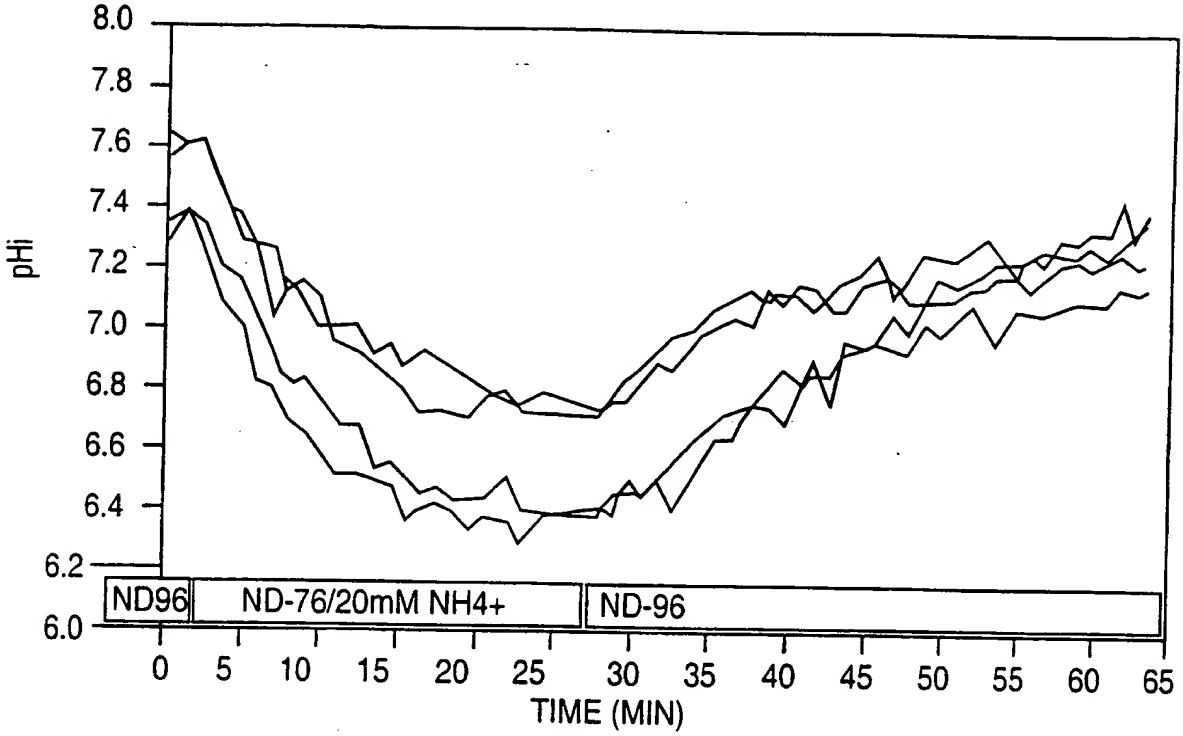


FIG. 29A

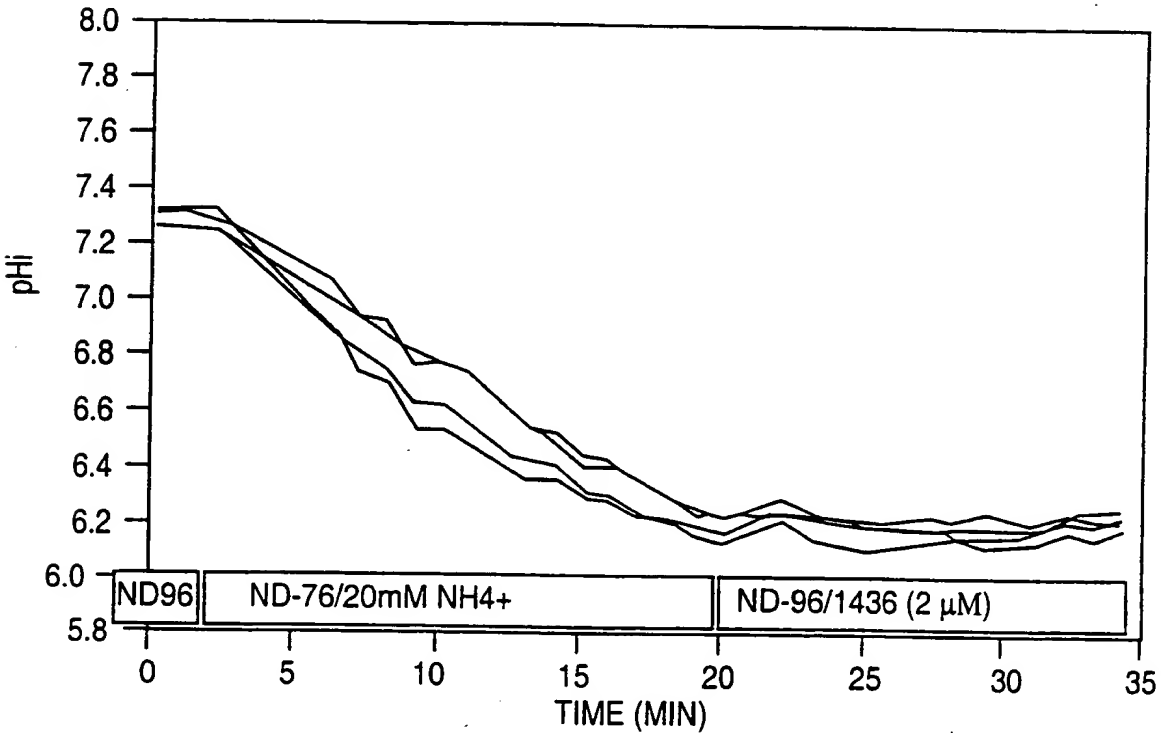


FIG. 29B

	ΔpH_i (UNITS)	$\%pH_i$ RECOVERY (AFTER 15 MIN)	dpH_i/dt (min^{-1})
CONTROL (n=3, s.d.)	-0.83 ± 0.08	63 ± 11	0.034 ± 0.002
1436 (2μM) (n=4, s.d.)	-0.97 ± 0.14	6.6 ± 4.9	-0.004 ± 0.003*

FIG. 30

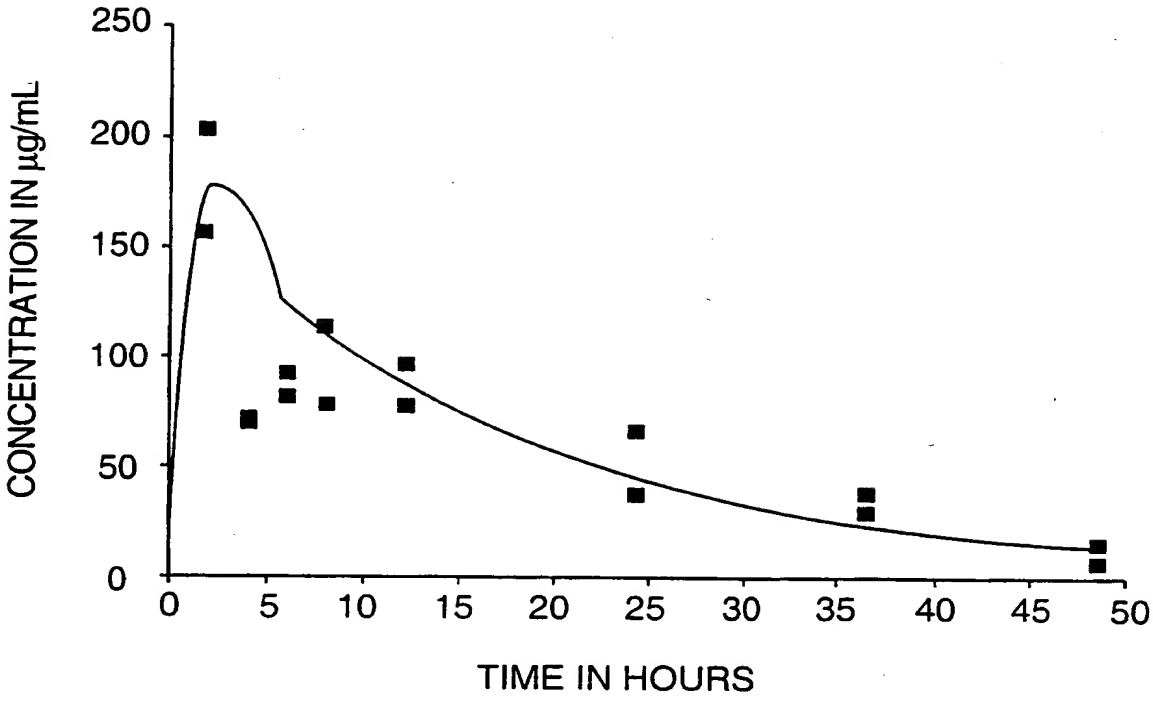

250
200
150
100
50
0
CONCENTRATION IN µg/mL
0
5
10
15
20
25
30
35
40
45
50
TIME IN HOURS

FIG. 31

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19595

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/575

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S.L. ALPER: "Shark aminosterols inhibit murine AE2 and AE1 and the endogenous Na/H exchanger in Xenopus oocytes" J. AM. SOC. NEPHROL., vol. 7, no. 9, September 1996, page 1249 XP002059790 see abstract	1-14
X	WO 95 24415 A (MAGAININ PHARMA) 14 September 1995 see page 4, line 9-14; claims	16-18,20
E	WO 97 44044 A (MAGAININ PHARMA) 27 November 1997 cited in the application see claims & US 60/017,627 (1996)	19

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 1998	01/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Orviz Diaz, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19595

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
P,X	WO 96 40151 A (MAGAININ PHARMA) 19 December 1996 cited in the application see page 126 - page 128 & US 08/474,799 (1995)	20
P,X	WO 96 40728 A (MAGAININ PHARMA) 19 December 1996 cited in the application see page 7; claim 20 & US 08/487,443 (1995) & US 08/483,057 (1995)	20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/19595

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9524415 A	14-09-95	AU 6397494 A	26-09-94
		AU 8010194 A	25-09-95
		CA 2185123 A	14-09-95
		EP 0688333 A	27-12-95
		EP 0749437 A	27-12-96
		JP 8507527 T	13-08-96
		JP 9509959 T	07-10-97
		US 5637691 A	10-06-97
WO 9744044 A	27-11-97	NONE	
WO 9640151 A	19-12-96	AU 6152896 A	30-12-96
WO 9640728 A	19-12-96	AU 6334996 A	30-12-96